THE USE OF A ROOT BIOASSAY TO INDICATE THE PHOSPHORUS STATUS OF FOREST TREES

Morag A. McDonald

Ph.D.

University of Edinburgh,

Department of Forestry and Natural Resources

December 1987

DECLARATION

I declare that the work reported in this thesis, except where stated otherwise, is the result of my own original research, and that no part has been presented for a higher degree.

Morag A. McDonald

December, 1987

ACKNOWLEDGEMENTS

This work was supported by a Natural Environment Research Council Studentship. The sites were made available by the Forestry Commission, who also supplied the seedlings. Additional thanks are due to the research staff at Keilder Forest and Charlie Taylor at the Northern Research Station. Facilities were made available by the Department of Forestry and Natural Resources at the University of Edinburgh and the Institute of Terrestrial Ecology at Merlewood. Thanks go to many people at both establishments for help and advice, in particular John Dighton, Doreen Howard and Allan Nelson at Merlewood; Andy Gray and Dave Haswell in the Department of Forestry and Natural Resources and members of the Molecular Biology Department in the University for the use of their scintillation counter, and help with same.

Above all, I am indebted to my supervisors, Dr. D. C. Malcolm and Dr. A. F. Harrison for their continued advice and help throughout this project, and for reading the drafts at the end; and to my family and friends for all their support.

ABSTRACT

A bioassay for determining the phosphorus status of plants, based on the rate of uptake of ^{32}P-labelled phosphorus by roots from a standard solution has been applied to excised roots from mature forest trees. Preliminary results showed a negative relationship between root uptake of P and quantities of P fertiliser previously applied, and to tree height. These results suggested that the bioassay detected a phosphorus status in trees not identifiable from needle analysis, the usual method of assessment, and may therefore provide an indication of the fertiliser demand in mature forest stands.

The objectives of this study were; to investigate the potential of the bioassay as an indicator of trees' nutritional status; to investigate factors influencing variations in root response; to assess the degree to which the bioassay may integrate the factors influencing trees' nutritional status; and from these investigations, to make an assessment of the potential of the bioassay as a field predictor of fertiliser requirement.

Experimental work involved both controlled experiments and field studies. Seasonal variation was examined in a polestage Sitka spruce stand (planted 1962), and greenhouse pot experiments examined the effects of moisture and temperature under controlled conditions. Variations in root uptake of ^{32}P were obtained over a period of thirteen months, but the effects of moisture and temperature were not clear.

Sampling in stands growing on spaced-furrow ploughing showed spatial variations in bioassay responses between furrow, ridge and flat. Greenhouse split-root pot experiments showed that within plant variation was of lesser importance in the interpretation of the bioassay results.

It was assumed from these experiments that the bioassay integrated the factors influencing nutrient availability and hence the overall status of the tree. Thus, times and positions of sampling are suggested to standardise sampling procedure.

However, the variability of the bioassay results was such that critical values of ^{32}P were not obtained which would predict a response to fertiliser application. Hence, its use as a routine field tool may be restricted, although further work is necessary to realise the extent of the variability over several sites in several years.

The bioassay proved to be a quick and easy technique, and modifications are suggested to further facilitate its use.

Throughout the project, the bioassay as an indicator of phosphorus status was consistent - demonstrated by a greenhouse pot experiment involving different applied rates of phosphorus fertiliser, but also in the field studies.

The bioassay is not as robust as conventional techniques available to assess nutrient status, because of its use of fresh root samples. However, this problem is offset by the greater ease of sampling, and the sensitivity of the technique in determining nutrient status.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

Afforestation of upland areas of Britain has been widespread over the last sixty years, using a variety of coniferous species. Early plantings tended to match species requirements with site conditions. However, the choice of species proved to be limited under the conditions prevailing at these sites, and by far the most successful proved to be Sitka spruce (*Picea sitchensis* (Bong.) Carr.) or, less often, lodgepole pine (*Pinus contorta* Dougl.). Plantations of these species have been established on a wide range of soil types, mainly peaty iron-pan podsols, peaty gleys and a range of peat soils, of varying fertility.

While potentially high yielding, Sitka spruce, especially, is nutritionally demanding, and the value of supplementing soil nutrient reserves with fertilisers was one of the first lines of approach to improving tree growth on these sites. The earliest manuring trials in the 1920's established the beneficial effects of phosphorus (P) fertilisers in particular (Zehetmayer, 1954). The first P fertiliser employed was basic slag, which had been used before the 1914-18 war by Sir John Stirling Maxwell at Corrour, Invernesshire, and was applied in research plots and then in general practice. Use of basic slag later gave way to ground mineral phosphate, which was finely ground phosphate rock mainly imported from North Africa. In South Wales and South-West England triple superphosphate was also used in the post-war period and is still preferred for hand application in parts of South-West England. Following the early trials, phosphatic fertilisation at planting became common practice in British forestry, these being the only fertilisers used until the late 1950's. In the mid-1960's unground phosphate rock was introduced which led to a great increase in the treatment of existing forests from the air (Binns, 1975). This, combined with the fact that afforestation increasingly was confined to poorer, less fertile sites as land for

afforestation became scarcer, led to an increased usage of phosphorus, and other inorganic fertilisers (Binns, 1975; Mayhead, 1976). In particular, the area of forest top-dressed each year has increased and large-scale operations seem likely to continue. This has increased the importance of being able to assess as precisely as possible the fertiliser requirement of a given stand, and when it is needed. Fertilising before it is needed is wasteful - the United Kingdom and Europe are wholly dependent on imports for supplies of phosphorus, a situation which is unlikely to change since rock phosphate deposits in the countries of the European Community are of very low quality (C.A.S., 1978). United Kingdom expenditure on imported rock phosphate rose from about £11 M a year in the early seventies to about £50 M per year from 1974 onwards (Bowman, 1978) and about 85 percent of P imported is for use in agriculture and forestry as fertilisers.

Conversely, waiting until nutrient deficiency is obvious means a loss of timber and revenue. The role of P in plant metabolism is complex, and a shortage is difficult to diagnose until the deficiency is acute, which may take several years to manifest. Phosphorus biochemistry has been dealt with by several authors including Arnon (1953) and Williams (1978) but in plants, it is a vital structural component of nucleic acids, nucleoproteins, phytin, phospholipids, adenosine triphosphate and numerous other phosphorylated compounds. Thus, P plays a vital role in many processes, but particularly in energy transfer. Hence, a deficiency will always result in restricted growth to some degree, and P is of special importance in root development and in the ripening of seeds and fruit (Binns *et al.*, 1980).

The efficient use of phosphorus fertiliser in forestry, before shortages are apparent, demands a method of predicting, for a given stand, the degree of response that will occur to a given fertiliser application (McIntosh, 1984). Several reviews of the various methods available to assess nutrient status exist (e.g. Morrison, 1974; Weetman, 1981; Miller, 1982) but it is generally concluded that a universal testing method, which is fast and accurate and which will diagnose fertiliser requirements, and the probable response to fertilisation, is yet to be found.

The forester has relied on the analysis of current years needles for phosphorus and other nutrients as a measure of trees' nutritional status (Everard, 1973). However, Van den Driessche (1974) concluded that foliar nutrient concentration was useful as a diagnostic tool, to decide which nutrient(s) is deficient in a stand growing poorly, or in check, but predictive use, to estimate responses from fertiliser application is difficult. Before canopy closure, during the first nutritional stage defined by Miller (1981), when growth is dependent on soil nutrient concentrations, foliar analysis has proved to be a reasonable indicator of the tree's nutritional status. After canopy closure, however, the capture and retention of atmospheric inputs is more efficient and nutrients are recycled within the tree, and up to 50-60 percent of the nutrient requirements of the tree for new growth can be satisfied by retranslocation (Miller, 1984). At this stage because of the role of P in energy transfer, greater concentrations occur in younger, more active tissues, that is, the distal shoots of the stem which are being sampled. Hence, foliar analysis of these shoots may not represent the overall nutrient status of the tree. Foliar analysis is particularly inadequate in closed-canopy, pole-stage stands which were established without the use of fertilisers, either because of inherent fertility of the sites afforested or because their establishment pre-dated current fertiliser practices. It is thought that fertilisers may increase the productivity of such sites, and many other established forests, but nutrient concentrations have all been above the levels associated with acceptable growth rates (Binns *et al.*, 1980), and have shown little or no relationship with growth or fertiliser response (McIntosh, 1984), especially with Sitka spruce. There is therefore a need for a test to unequivocally establish fertiliser needs and probable responses in forest stands.

The importance of radioisotopes to fertiliser usage in agricultural research has long been established. Problems embracing efficiency of fertiliser applications, comparison of fertiliser materials, rate, time and placement of fertilisers, influence of soils and crops on fertiliser utilisation and soil fertility evaluation were considered with the use of radioisotopes as early as 1947 (Hendricks and Dean, (1947); Spinks and Barber, (1947)). Almost all of these experiments have dealt with phosphorus

fertilisers, one reason for this being the desirable characteristics of the isotope ^{32}P; which is a high energy radionuclide with a short half-life. Uptake of ^{32}P by plant root systems over short periods of time have also long been recognised as efficient, economical methods of studying the phosphorus status of plants (e.g. Hoagland and Broyer, 1936; Humphries, 1950; Epstein *et al.*, 1963; Chapin and Bloom, 1976). Bowen (1971) used such a bioassay to detect phosphate deficiency in pine, wheat and ryegrass seedlings long before deficiency symptoms became apparent, relating the internal phosphorus status of the plant to its uptake of ^{32}P from a phosphate solution.

The rate of uptake of an ion has often been shown to be a function of its internal status of that ion. 'Starving' plant roots of phosphorus has been shown in *in vitro* studies to increase the rate of uptake of the phosphate ion (Drew *et al.*, 1984; Lee, 1982) or root affinity for the ion (Cartwright, 1972). The response has been shown to be very rapid and many plants can probably therefore react to short-term fluctuations in the root surface ion concentration by this sort of kinetic change, probably the result of synthesis of new carriers in the membrane or possibly of allosteric changes in existing carriers (Fitter and Hay, 1987).

Earlier evidence suggested that these changes might be determined by fluctuations in cytoplasmic ion concentrations, but this was based on whole root ion concentrations (Glass, 1978). Recently the development of NMR (neutron magnetic resonance) techniques has allowed the measurement of ion concentrations in specific cell compartments, and it now appears that over a wide range of external P concentrations, the inorganic P concentration of the cytoplasm is almost unaltered, while that of the vacuole changes markedly (Lee and Ratcliffe, 1983). In the light of this, it is thought that the flux across the tonoplast may regulate carrier behaviour.

However, whatever the external concentration and the rate of uptake, the internal concentration of the ion will depend on the rate at which it is incorporated metabolically (Fitter and Hay, 1987). Therefore, any environmental factor affecting the metabolic rate of a plant will affect its ion uptake. This phenomenon is given major consideration throughout this study.

Bowen recognised that his bioassay would be of particular value with slow growing species not readily producing visible deficiency symptoms. Harrison and Helliwell (1979) applied the technique to birch (*Betula verrucosa* Ehrh.) and sycamore (*Acer pseudoplatanus* L.) seedlings to assess the availability of phosphorus in soils. They found that the amount of ^{32}P taken up by birch seedlings was largely governed by the availability of phosphorus in the previous rooting environment. This was true for four soil phosphorus properties that they assessed - isotopically exchangeable P, extractable P, total P and soil phosphatase activity. This was less marked for sycamore seedlings, which they attributed to the higher nutrient reserves of sycamore seed. Harrison and Helliwell concluded that the bioassay was a reliable indicator of the phosphorus status of the seedlings, as suggested by Bowen (1971) and that it could be used to assess the availability of phosphorus in soils, and to integrate the degree to which the plant phosphorus demand has been satisfied.

The rapidity and precision of the bioassay, and the fact that its precision increased with decreasing soil fertility (the reverse of most chemical methods used) led Harrison and Helliwell to consider using the technique for determining the phosphorus status of soils for a particular crop, such as forest trees. An investigation into the P-nutrition of trees, using the bioassay, and relating the results to tree height and foliar concentration was carried out by Dighton and Harrison (1983). Excised roots were studied instead of intact seedlings. Earlier studies by Hoagland and Broyer (1936) and Humphries (1950) used excised roots and found uptake similar to that by intact plants. Bowen and Theodoru (1967) and Michalik (1974) showed P uptake by excised root tissue to be metabolic and this was corroborated by Dighton and Harrison (1983) who found ^{32}P uptake to be blocked in the presence of potassium cyanide, a metabolic inhibitor. When the bioassay was applied to excised roots of field grown lodgepole pine (*Pinus contorta* Dougl.) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.), the results were consistent with those reported for pot-grown seedlings by Harrison and Helliwell (1979). The ^{32}P uptake figures were negatively related to applied fertiliser levels and tree height, but less well correlated with foliar

concentrations. It was considered that the bioassay may provide a means of predicting fertiliser requirements of trees.

On the basis of these preliminary results this study was initiated with the objectives of; (1) investigating the potential of the bioassay as an indicator of trees' nutritional status; (2) to investigate factors influencing variation in root responses, and (3) assessing the degree to which the bioassay may integrate the factors influencing trees' nutritional status.

From these investigations, with a better understanding of factors limiting the interpretation of the bioassay results, an assessment is made of the potential of the bioassay as a field predictor of fertiliser requirements.

The procedures for using the bioassay and modifications tested to enhance practical use are considered in Chapter 2.

The inverse relationship between applied P fertiliser and ^{32}P uptake, under controlled conditions in intact seedlings is substantiated in Chapter 3.

A field sampling programme to investigate seasonal variation, and controlled experiments to examine the principal factors thought to give rise to seasonal variation are presented in Chapter 4.

A study of spatial variation in ^{32}P uptake within the forest, and in controlled experiments to examine variability within the tree is discussed in Chapter 5.

Finally, using the results from these studies, Chapter 6 assesses the general potential of the ^{32}P bioassay as a practical and research tool and outlines the desirable direction of further studies.

CHAPTER 2

The Phosphorus Bioassay

2.1 Introduction

The papers referred to previously (Harrison and Helliwell, 1979; Dighton and Harrison, 1983) outline the method which forms the basis of this research. The first paper describes the method in outline and a later research and development paper (Harrison, Dighton and Smith, 1984) presents a more detailed account of the methodology involved.

Here, the method is summarised as it is applied to the sampling procedures used. Also, several modifications to the original bioassay are proposed to facilitate its use.

2.2 Methods

2.2.1 Root sampling

When the bioassay is applied to pot-grown seedlings, plants are removed intact from the pot and surplus soil is shaken off. Each plant is appropriately labelled and the root systems carefully washed under running tap water. The plants are then stored in a cool room (4 ^{o}C), under moist tissue papers, until execution of the bioassay on the following day.

When applied to roots from forest stands, roots are teased from the 0-5 cm soil (LFH) horizons, and labelled. The roots are collected from random positions on the 'flat' forest floor (See Chapter 5) within an 'assessment plot' in the treatment plot. Intact segments of 10-20 cm axial length and 0.5-2.0 mm diameter, usually mycorrhizal, are transported between moist tissue papers in plastic trays to the laboratory. These roots are then washed carefully under running tap water and kept under moist tissue papers in a cool room (4 ^{o}C) until assay the following day.

2.2.2 Bioassay Procedure

Excised roots, or the root systems of intact seedlings are placed in a 5 x 10^{-4} M calcium sulphate solution for 30 minutes. This immersion is necessary to remove phosphorus from the free space of the root as unlabelled phosphorus diffusing into the

^{32}P labelled solution would alter the specific activity in an indeterminate way. Also, calcium ions may stimulate phosphorus uptake and accumulation in the root (Miller *et al.*, 1972) and help to maintain root cell membrane integrity.

Roots are then transferred to a solution containing 5×10^{-4} M calcium sulphate, 5×10^{-6} M potassium dihydrogen phosphate and about 1 MBq ^{32}P l^{-1} as orthophosphate, maintained at 18 °C by a water bath. The roots are left in the solution for a 15 minute period.

Prior to immersion of the roots in the radioactive solution, 0.5 ml sample standards of the solution were added to 15 ml of distilled water in low-potassium glass counting vials and counted by Cerenkov light in an automatic Intertechnique SL 3000 liquid scintillation spectrometer. Cerenkov radiation has an energy spectrum in the ultra-violet/visible range and can be measured directly, from aqueous solution, without organic fluors (Chapin and Holleman, 1974). Cerenkov radiation occurs with β - emitting radionuclides showing emission energies above 1 MeV, such as ^{32}P which has a maximum emission energy of 1.71 MeV. Organicfluors are expensive and so, when large numbers of samples are involved, counting by Cerenkov light is the most economical method applicable. Organic fluors may also damage cell membranes and so their use should be avoided (Harrison *et al.*, 1984).

After the 15 minute period in the radioactive solution, the roots are immediately transferred to a large beaker through which tap water is circulating to remove unabsorbed ^{32}P from the root surfaces. After five minutes washing, the roots are removed and fresh weight samples of about 200 mg taken from the terminal ends of lateral roots. The samples are placed in 15 ml distilled water in scintillation vials and ^{32}P counted under the same conditions as above. After counting, each root sample is removed from its vial, blotted on tissue paper, and weighed, and the ^{32}P remaining in the vial recounted under identical conditions. This second count is of any further ^{32}P which was not metabolically absorbed by the root and which diffused from the root surface into the water of the vial. The second count is subtracted from the first count

to give values for metabolic uptake by the roots.

The values thus obtained are counts of ^{32}P per minute and have to be corrected for background radiation levels and decay of the ^{32}P over time. Background count is measured in vials of distilled water, and subtracted. Decay is allowed for using the time elapsed between root immersion in the radioactive solution and when counting starts. The time at which the vials are recounted, without roots, is also noted, so that a further elapsed time can be calculated. The ^{32}P solution can be re-used for subsequent batches of roots, and new elapsed times recorded, but allowance must be made for the reduction in the rate of uptake of ^{32}P and phosphate due to a lower concentration of the ^{32}P labelled in the solution. The rate of uptake is linearly related to concentration over the low ranges of concentration involved (Harrison *et al.*, 1984) and so, increases in the estimates of ^{32}P uptake by the roots are made from the ratio of the ^{32}P activity in the solution before any roots were immersed to activity before the particular batch of roots in question.

The counts obtained from the spectrometer have also to be modified for the counting efficiency of the system, which assumes that the vials contain only homogenous solutions, and therefore does not allow for the effects of the roots on radioisotope emission.

Colour in the root samples 'quenches' the pulses produced by photomultipliers in the spectrometer by reducing the intensity of the light flash emitted by the radioactive root. This has the effect of displacing the emission spectrum (Figure 2.1), the extent of which is gauged from the function of the 'channels ratio', between two counting channels. The counting efficiency is linearly related to the channels ratio by the equation :

$$y = a X + C$$

where X is the channels ratio. The relationship is evaluated by adding 10 microlitres of a 1 microcurie per ml calibrated ^{32}P solution to vials containing varying amounts of colour quenching substances (e.g. NaOH extracts of roots). The vials are then

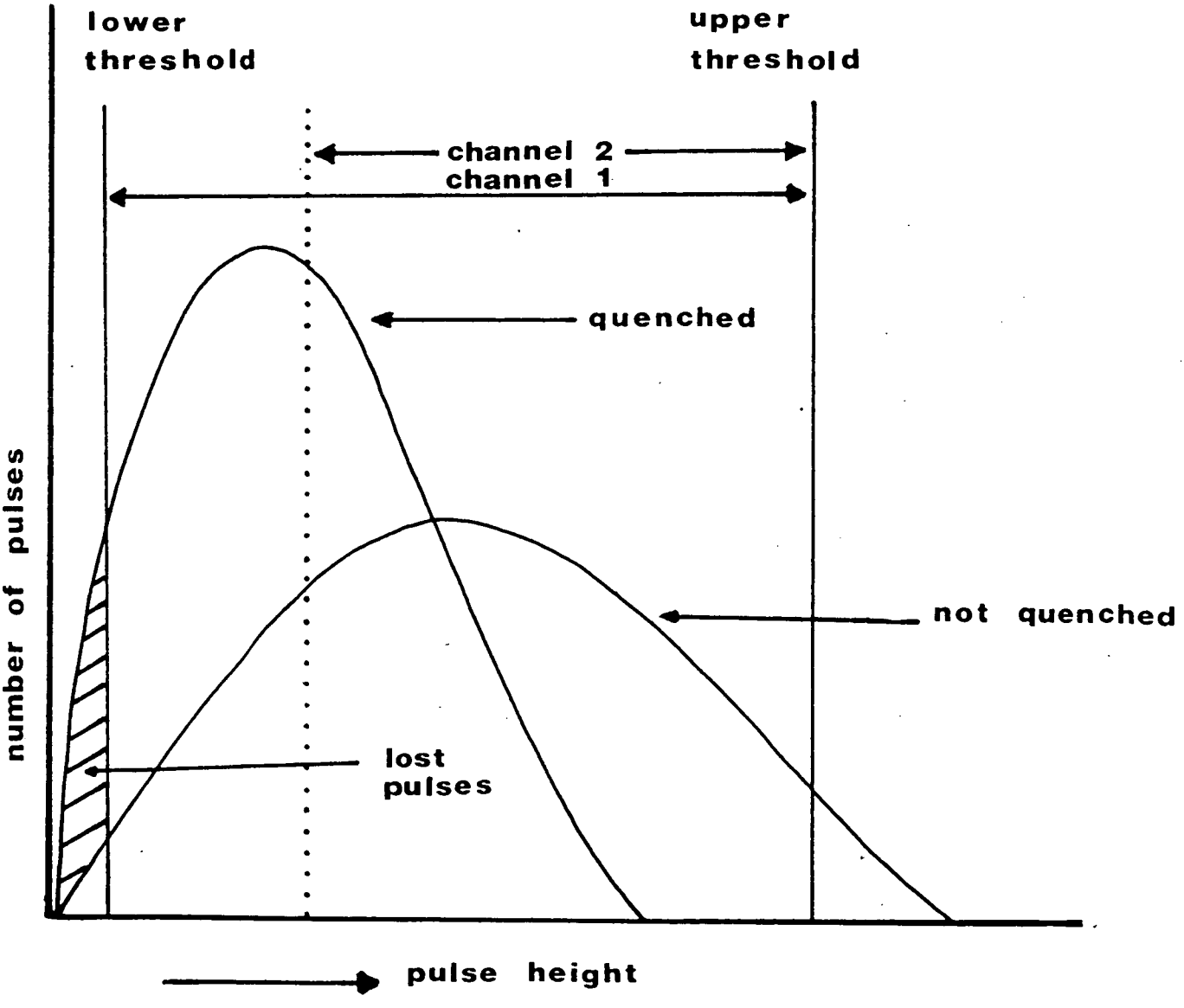


FIG. 2.1: The effect of root quench on spectrum shape.

counted in two channels, and the channels ratio calculated. The counting efficiency is calculated from the counts per minute estimate of the channel with the wider spectrum after allowing for the ^{32}P decay and the background count in that channel, and dividing by the known amount of activity, in disintegrations per minute, present in 10 microlitres of the standard.

This equation is not valid when there is root biomass in the vial, and so, to allow for this physical quenching effect, a stratified random sample of the roots is taken, after counting, and digested in a concentrated nitric-perchloric-sulphuric acid mixture (in the ratio of 10:2:1 respectively) as no significant losses of ^{32}P occur when this acid mixture is used (Allen *et al.*, 1974). Digestion takes approximately 1.5 hours, after which time, the digested root is made up to a solution of 15 ml with distilled water, and transferred to a counting vial. The digests are counted under the same conditions as the root samples. The equation to allow for the effects of root quench takes the form of:

$$Y = aX + bR + cR^2 + d$$

where X = estimated ^{32}P activity in disintegrations per minute
before allowance for root quench.

R = root (moist) weight in mg.

The relationship is derived from a multiple regression from the estimated ^{32}P content of the root selected for digestion (i.e. counts of vials with roots minus counts of vials without roots, both after correction for background, counting efficiency and decay), root weight, and the estimated ^{32}P activity in the digest (after allowance for background, counting efficiency and decay).

The allowances for background, decay, counting efficiency, root quench and also the root weight (moist) are all calculated using computer programmes (Harrison *et al.*, 1984) and the final values, of disintegrations per minute, are expressed as pg ^{32}P uptake per mg of root. A simplified flow chart of the bioassay activities is presented in Figure 2.2.

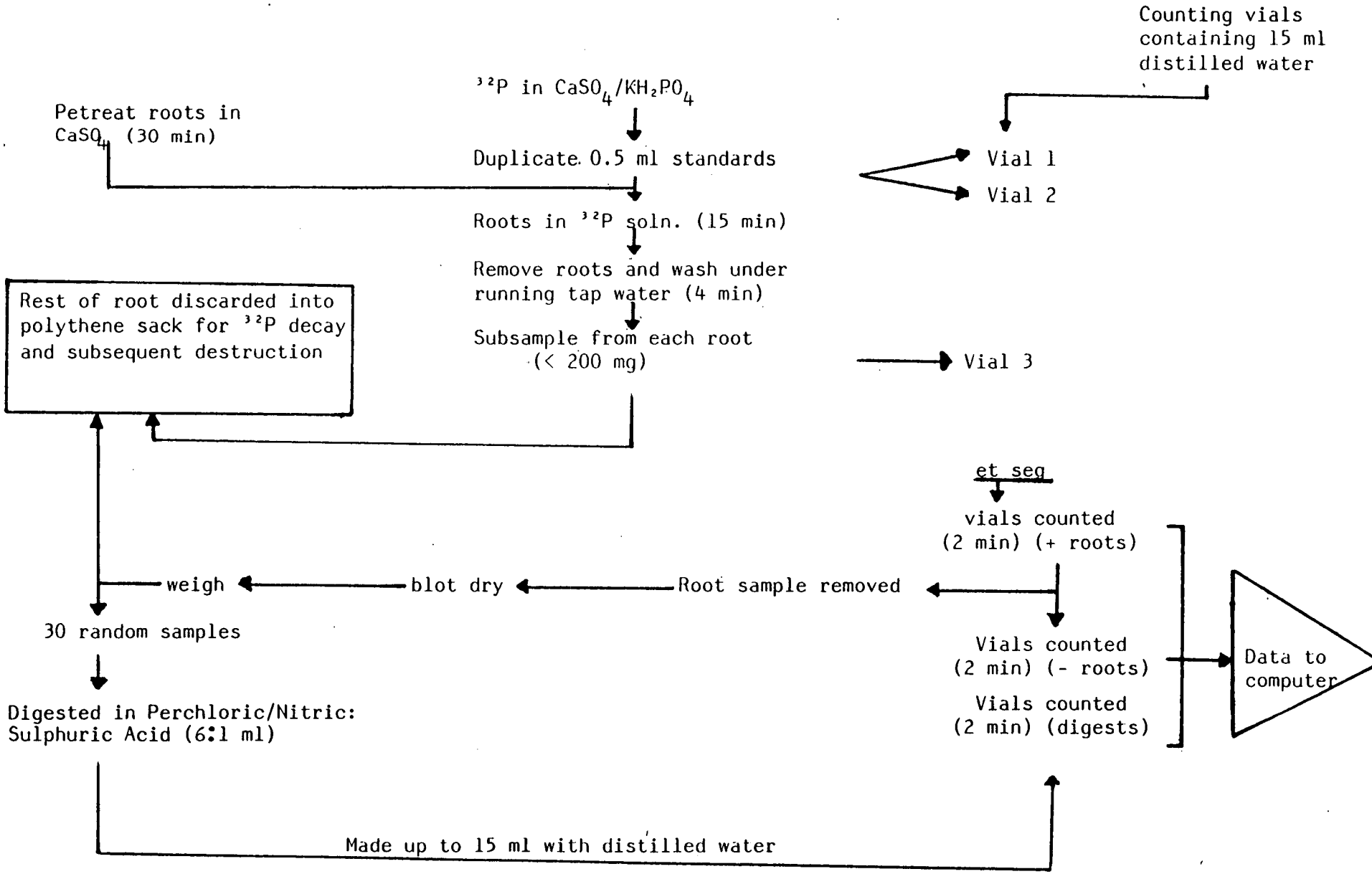


FIG. 2.2: Flow Chart of Bioassay Activities (from Harrison et al., 1984)

2.3 Modifications

2.3.1 Sample size

In the original development of the bioassay as a field tool, fifty roots were taken from a replicate block for analysis (Dighton and Harrison, 1983). These consisted of five root samples from each of ten randomly selected trees per block.

However, this results in a very extensive sampling programme if several replicate blocks are involved, and one which may not be practical for repeated sampling. It is also difficult to identify the parent tree of a sampled root in a mature forest stand where rooting is extensive over the forest floor.

Therefore, to examine the feasibility of restricting the sample size, a data set from Shin Forest, Sutherland, from an earlier bioassay application (Dighton and Harrison, 1983) was examined. This Forestry Commission experiment, planted in 1972, consisted of three replicate blocks of the following treatments:

(i) No fertiliser at all

(ii) P at planting (50 kg P ha^{-1})

(iii) P at planting and P (50 kg P ha^{-1}) in June 1982.

From each of the nine blocks in the experiment, five root samples from nine randomly selected trees were collected. In this experiment, each root was subsampled twice during the bioassay after immersion in the ^{32}P solution, giving the data the format as laid out in Table 2.1(i). The lack of orthogonality is due to spurious results in some treatments. If the attribution of root sample to parent tree is considered dubious in some stands, then these data may be regarded as 45 roots sampled per replicate block.

Ten roots per replicate block were considered feasible for an individual sampling programme, while still retaining sufficient degrees of freedom to determine significant statistical differences. Therefore, the values for ten roots from each replicate block were randomly extracted from the data, thus constructing a new data set with ten roots per replicate block, twenty values in all, as laid out in Table 2.1(ii).

Table 2.1 (i) Format of the original Shin data
(ii) Format of the randomly extracted data for re-analysis

(i) Block	Tree	1	2	3	4	5	6	7	8	9	Totals
Trt	A	10	10	10	10	10	10	10	6	10	86(90)
i	B	10	10	10	10	10	10	10	10	10	90
	C	4	8	10	10	6	1	10	6	8	63(90)
	D	10	10	10	10	10	10	10	10	10	90
ii	E	10	10	10	10	10	10	10	10	10	90
	F	10	10	10	10	10	10	10	10	10	90
	G	10	10	10	10	10	10	10	10	10	90
iii	H	10	10	10	10	10	10	10	10	10	90
	I	10	10	10	10	10	10	10	10	10	90
											779

(ii) Block			Totals
Trt	A	Ten randomly selected roots	20
i	B	"	20
	C	"	20
	D	Ten randomly selected roots	20
ii	E	"	20
	F	"	20
	G	Ten randomly selected roots	20
iii	H	"	20
	I	"	20
			180

The two values obtained for each root sampled are not, however, independent, and with only one degree of freedom, the proportion of variation attributable to these values cannot be defined. Therefore, the average value is calculated and used in the statistical analysis thus giving ten and forty-five values per block respectively for the two data sets.

Both data sets were analysed by analyses-of-variance using the statistical computing package, GENSTAT. The ANOVA tables are presented in Table 2.2(i) and (ii). Summary statistics for the two data sets are presented in Table 2.3(i) and (ii).

The statistical analysis indicates that the two data sets are consistent. There is slight variation between the mean values, but they lie within the appropriate 95 % confidence intervals and can be considered similar. The error term shows an increase with a reduction in size of the data set but is less than 10 % of the mean value.

The ANOVA indicates that, with a reduced data base, a greater proportion of variation is accounted for by the treatment effect than the effects of replication. However, in both cases, variation between blocks, within treatments was insignificant.

On the basis of these analyses, then, it was considered that results for a smaller sample size were consistent with the larger data base, and considering the desirability in practice of reducing the sample size, it was concluded that ten roots are sufficient to detect treatment differences, especially if replicate blocks are available and if the degree in variability within other experiments remains about the same.

2.3.2 Expression of ^{32}P uptake in relation to fresh or dry weight

2.3.2.1 Introduction

After the root subsamples have been counted for ^{32}P content, they are removed from their vials, blotted dry and their fresh weight recorded (Section 2.2.2). ^{32}P uptake is hence given as; pg ^{32}P mg^{-1} fresh root weight. Fresh weight is a more practical parameter of uptake than dry weight, in terms of ease of measurement over a short period of time.

Table 2.2 (i) ANOVA for original Shin data
(ii) ANOVA for randomly extracted data

(i) Source of Variation	DF	SSQ	MSQ	VR
Treatment. Block Stratum				
Treatment	2	6751426	3375713	25.43***
Residual	6	796384	132731	1.42 ns
Total	8	7547809	943476	
Treatment. Block. Root Stratum	396	35717248	93501	
Grand Total	404	43265056		
Grand Mean	**442**			
Total No. of Observations	**405**			

(ii) Source of Variation	DF	SSQ	MSQ	VR
Treatment. Block Stratum				
Treatment	2	1911675	955838	80.08***
Residual	6	71613	11936	0.41 ns
Total	8	1983288	247911	
Treatment. Block. Root Stratum	81	2345744	28960	
Grand Total	89	4329032		
Grand Mean	**418**			
Total No. of Observations	**90**			

TABLE 2.3: (i) Summary statistics for original Shin data
(ii) Summary statistics for randomly extracted data.

(i)

Treatment	N	$\bar{x}$	s.d.	s.e.	95%C.I.
i	135	559.9	462.4	42.0	83.2
ii	135	469.6	225.5	19.4	38.39
iii	135	251.9	173.6	14.9	29.5

(ii)

Treatment	N	$\bar{x}$	s.d.	s.e.	95 % C.I.
i	30	558.3	231.5	42.3	86.5
ii	30	477.6	119.4	21.8	44.6
iii	30	216.8	124.5	22.7	46.5

Fresh weights of plant tissue are, however, generally regarded as being highly variable, and dry weights are usually preferred as an expression of plant biomass.

An investigation was carried out to assess the significance of results from the bioassay when expressed as uptake of ^{32}P mg^{-1} of both the fresh and the dry weight of root.

2.3.2.2. Methods

Thirty root samples were collected, as described in Section 2.2.1, from the Forestry Commission nutrient deficiency garden at Leadburn, near Edinburgh in May 1984 (Section 5.2) from each of plots 9, 13, 20 and 24 (Appendix 5A). These Sitka spruce and lodgepole pine plots had been either fertilised with phosphorus or non-fertilised. The bioassay was carried out, as described in Section 2.2. However, instead of discarding the roots after weighing, all the roots except 32 digested to enable correction for quench, were oven-dried at 85 ^{o}C to constant weight. Another digest series was carried out on the dried roots.

The raw data from the scintillation counter was computed, using both the dry and the fresh weight of the roots and ^{32}P uptake hence expressed as pg ^{32}P mg^{-1} of both fresh and dry root.

2.3.2.3 Results and Discussion

Values for ^{32}P uptake expressed as pg ^{32}P mg^{-1} fresh and dry root are given in Table 2.4 (i) and (ii) respectively.

Analyses-of-variance, using the statistical computing package, SPSS, showed heterogeneity of variances requiring a $\log_{10}$ transformation. ANOVA tables for the transformed data are presented in Table 2.5(i) and (ii).

Duncan's Multiple Range tests ($P \leqslant 0.05$) showed that, for both data sets, neither the two fertilised nor non-fertilised plots were significantly different.

Therefore, whether uptake is expressed in terms of dry or fresh weight, the results obtained are similar, in that the same treatment differences are detected. Furthermore, the ANOVA tables indicate that greater variability is introduced if uptake

Table 2.4 (i) pg ^{32}P mg^{-1} fresh root weight means (s.e.) n = 30

(ii) pg ^{32}P mg^{-1} dry root weight means (s.e.) n = 26.

(i)

Sitka spruce	**+P**	36.17 (4.2)
	-P	268.73 (28.2)
lodgepole pine	**+P**	38.63 (7.9)
	-P	228.70 (28.9)

(ii)

Sitka spruce	**+P**	338.96 (74.5)
	-P	825.31 (122.4)
lodgepole pine	**+P**	332.58 (44.3)
	-P	1103.96 (307.6)

Table 2.5 ANOVA for transformed (log_{10}) root uptake data
(i) based on fresh weight (ii) based on dry weight.

(i)

Source of Variation	DF	SSQ	MSQ	F
Between Groups	3	21.37	7.12	71.46***
Within Groups	116	11.56	0.10	
Total	**119**	**32.93**		

(ii)

Source of Variation	DF	SSQ	MSQ	F
Between Groups	3	4.39	1.46	11.29***
Within Groups	100	13.00	0.13	
Total	**103**	**17.39**		

is expressed in terms of dry weight. For this data, a greater proportion of variation being attributable to 'within treatments' than 'between treatments'; illustrated by the greater proportion of the sum-of-squares accounted for. Hence, the overall treatment effect is lessened, as reflected in the 'F value', even though the treatment effect is still significant at $P \leqslant 0.001$. The error term is also increased when uptake is expressed in terms of dry weight.

The greater variability of the dry weights appears to be due to a higher moisture status in phosphorus deficient roots. In practical terms this effect is minimised as the roots are fully turgid when weighed, following immersion in the bioassay solutions. However, there may be a difference in root morphology as a consequence of this, such as thicker cell walls, the effects of which are not accounted for.

In view of the lesser variability involved and the greater ease of measurement, uptake will be expressed as pg ^{32}P mg^{-1} fresh root weight throughout.

2.3.3 Longevity of excised roots in storage

2.3.3.1 Introduction

During the initial applications of the bioassay to roots of forest stands, the roots were sampled as described in Section 2.2.1 and stored overnight in a cold store (4 ^{o}C) before bioassay. Dighton and Harrison (1983) carried out the bioassay within 72 hours of root excision.

Bowen (1967) noted that severance of roots induces immediate physiological changes in the roots of wheat and that such excision effects were detectable within 2 minutes. He found that there was a trend such that the effects of excision on total phosphate uptake were relatively greater after 15 minutes than after two minutes, but major changes occurred within 2 minutes. In general, phosphate uptake was retarded in all regions of the root.

If the major physiological changes occur very shortly after excision in forest tree roots, then uptake of phosphate may be reasonably constant over the period at which the roots are still metabolically active and in cold storage. If, however, uptake is not

constant, allowances will have to be made, or times standardised for which roots are stored after sampling before bioassay, even though roots from different treatments are processed in a random fashion.

Therefore, it was decided to investigate how the ^{32}P uptake by excised roots varied with time after excision at sampling.

2.3.3.2 Methods

An initial investigation examined the time periods relatively shortly after sampling.

Sixty root samples were collected from Plot 1 (Appendix 5A), phosphorus fertilised Sitka spruce, of the Forestry Commission nutrient deficiency garden at Leadburn, in August 1984. The roots were washed and placed in a cool room aunder moist tissue papers.

The shortest possible time at which it was feasible to assay roots after sampling was 4 hours, because of travelling from the field site and washing of the roots. After 4 hours, ten roots were randomly selected from the tray in which they were being stored, and assayed. The remaining roots were re-covered and left in the cool room. This procedure was repeated at periods of 8, 12, 24, 48 and 72 hours after sampling.

A similar investigation was conducted in May, 1985, at the same site, but on this occasion, ten roots were assayed at periods of 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, 103, 168, 376, 484 and 676 hours after sampling.

2.3.3.3. Results and Discussion

The preliminary investigation, in August, 1984, showed considerable fluctuation in the 72 hours after sampling, with a 3-fold increase in ^{32}P uptake over the first 48 hours (Fig. 2.3, Appendix 2). This was also true for the second investigation (Fig. 2.3, Appendix 2).

However, the second investigation showed that ^{32}P-uptake became more or less constant after 72 hours.

The implications of these investigations for the use of the bioassay appear to be

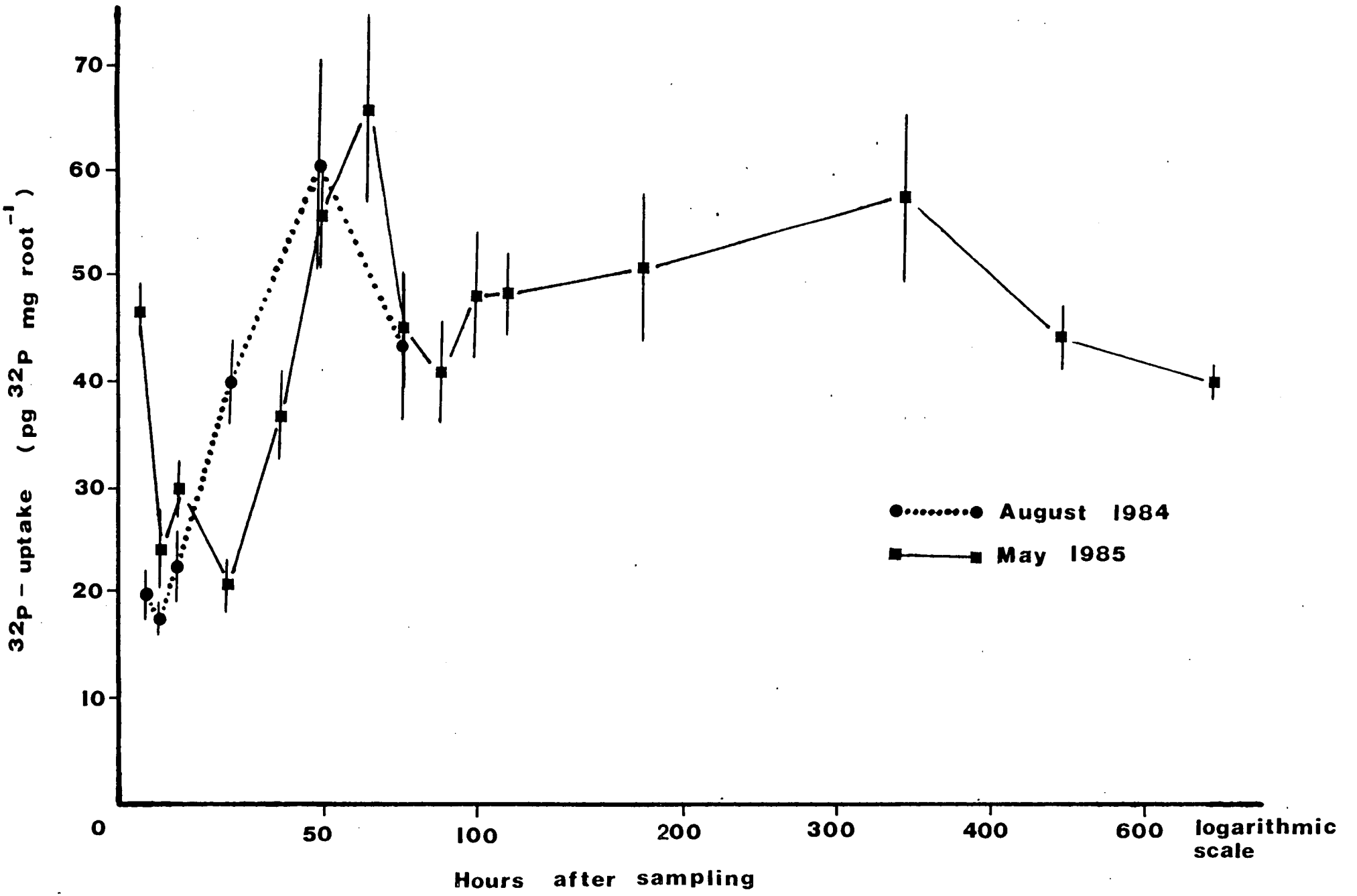


FIG. 2.3: Uptake of ^{32}P by roots stored for increasing time periods after sampling. ($\bar{x} \pm$ s.e., n = 10)

that, in a sequential sampling programme, the roots should be stored for approximately 72 hours before bioassay, to achieve a 'steady state' of uptake. However, ^{32}P uptake during the bioassay is a metabolic process (Dighton and Harrison, 1983) and so, uptake must be a function of the metabolic activity of the roots before excision. This activity is influenced by many factors, such as moisture and temperature and will be dependent upon the time and prevailing conditions at sampling. This is reflected in the different levels of uptake observed in the two investigations on roots from the same plot at different times.

In view of this, it was considered difficult to predict at what stage after excision a 'steady state' of metabolic activity would be achieved. Therefore, for the purposes of this research, the procedure was standardised in that roots collected were assayed 24 hours after excision. This time period was practicable for all the investigations carried out, and as shorter periods were not feasible, it was considered that this time interval would adequately represent the metabolic activity in the field. After this interval the variability of results increased considerably, and so, using longer intervals would increase the error for samplings. Also, particularly in the second sampling, there was little or no differences in results between 8 and 36 hours. The roots from different treatments were processed randomly over the 2-3 hours during which the bioassay was carried out.

CHAPTER 3

A Comparison of ^{32}P-Uptake by Different Species Grown under Different Rates of Phosphorus Fertilisation in a Pot Experiment.

3.1 Introduction

As stated in Chapter 1, an objective of the study was to determine conclusively whether there was differential uptake of ^{32}P from the bioassay solution by plants grown under different phosphorus fertiliser application rates.

The original demonstration of the bioassay was performed on deciduous species seedlings grown in sand culture (Harrison and Helliwell, 1979). To equate the situation to one of practical forestry, it was decided to grow coniferous species in pots of peaty soils with a fertiliser regime similar to the one imposed in establishing forest plantations, and to compare the results of the bioassay with conventional methods of assessing the P status of the plants, namely tissue analysis and productivity.

3.2 Methods

3.2.1 Planting Materials

Seedlings (1 + 0) of Q.C.I. Sitka spruce, Skeena lodgepole pine and Japanese larch were lifted from the Forestry Commission nursery on the Bush Estate, near Edinburgh, on 11 March 1984. They were stored overnight in a cold room (4 ^{o}C) in polythene sacks and then hand selected for uniformity and size and form, both above and below ground and planted in 5" square pots in horticultural peat on 12 March 1984. The pH of the peat, measured in water, was 3.10. On 15 March, four treatments were designated, plus a control, and twenty plants for each treatment, for each species, received phosphorus at rates equivalent to 5, 10, 20 and 50 kg ha^{-1} elemental phosphorus, the control received no P. This was applied at rates equivalent to 37.5, 75, 150 and 375 kg ha^{-1} of unground rock phosphate as top dressing. All plants, including the control plants, received potassium at a rate equivalent to 100 kg ha^{-1} elemental potassium as 200 kg ha^{-1} potassium chloride, also as a surface dressing. The standard Forestry Commission fertilisation rate at establishment was 50

kg ha^{-1} elemental P at that time and 100 kg ha^{-1} elemental K.

The plants were set up in twenty randomised blocks in a greenhouse, each block containing one plant for each treatment and a control of each species.

The plants were left, adequately watered, but with no further fertilisation over one growing season, until October 1984.

3.2.2 Measurements and Analysis

There was some mortality, particularly amongst the larch, and so, ten complete blocks were selected for analysis.

i) Root collar diameter

The root collar diameter has been shown to be highly correlated with both the dry weight, and the height, of the seedling (Aldhous, 1962; Freezailah, 1974). Therefore, as a measure of productivity, on the 12 October 1984, prior to harvesting of the seedlings, root collar dia meter was measured immediately above the soil level using a caliper divided to 0.01 mm.

ii) Phosphorus bioassay

The plants were harvested on 14 October, 1984 as described in Section 2.2.1 and the bioassay carried out as described in Section 2.2.2.

iii) Chemical Analysis

After washing the root systems after removal from the peat, sub-samples of root and shoot (approx. 2 g fresh weight) were removed from each plant and oven dried to a constant weight at 105 ^{o}C. Needles were then separated from the stem. The dried needles and root were then ground, separately, in a ball mill to approximately 0.5 mm mesh size.

Total N, P, K, Ca and Mg of the needles and roots were determined using a modified micro-kjeldahl digest (Allen *et al.*, 1974). 0.1 g of sample was accurately weighed into a pyrex digest tube to which was added 2 ml of 36N H_2SO_4 and 1 ml (dropwise) of 30 % (100 volumes) H_20_2. Tubes were placed in a heating block at 340 ^{o}C for 5 hours, after which all organic material had been destroyed and the

solutions had cleared. Samples were cooled, transferred to 50 ml volumetric flasks and made up to 50 ml with distilled water.Reagent blanks were run with each set of digested samples, 1 sample in 10 being duplicated to provide a check on reproduc ability.

Total N (as ammonium) was determined in solution using an automated colorimetric method employing the salicylate-dichloroisocyanurate reaction in the presence of nitroprusside (Crooke and Simpson, 1971). Total P (as phosphate) was determined in the solution using an automated colorimetric method employing the molybdate blue complex, ascorbic acid being used as the reducing agent in the molybdenum system (Murphy and Riley, 1962). Total K in the digested solution was determined directly by atomic emission and Ca and Mg by atomic absorption after addition of Lanthanum, using a Pye Unicam SP9 atomic absorption/emission spectrophotometer.

3.3 Results

The results were analysed by a 2-way analysis of variance using the statistical computing package, SPSS. Prior to statistical analysis, all data were checked for normality and homogeneity of variances. The results of the bioassay showed heterogeneity and required a $\log_{10}$ transformation before statistical differences could be established. The data presented in the text and appendices are, however, the original data. Orthogonal polynomials calculated by ASYST.

Where treatments and species effects were detected, Duncan's multiple range test was conducted on the treatment means for each species to establish significant differences.

i) Root collar diameter

The larch seedlings had the largest root collar diameters at the end of the experiment (Fig. 3.1, Appendix 3A), but there were no variations in the treatment means. The pine seedlings grown at the two highest phosphorus rates, P20 and P50, were larger than those from the P5 treatment, but were of a similar size to the other treatments, and in the spruce, plants from the control were smaller than those from the

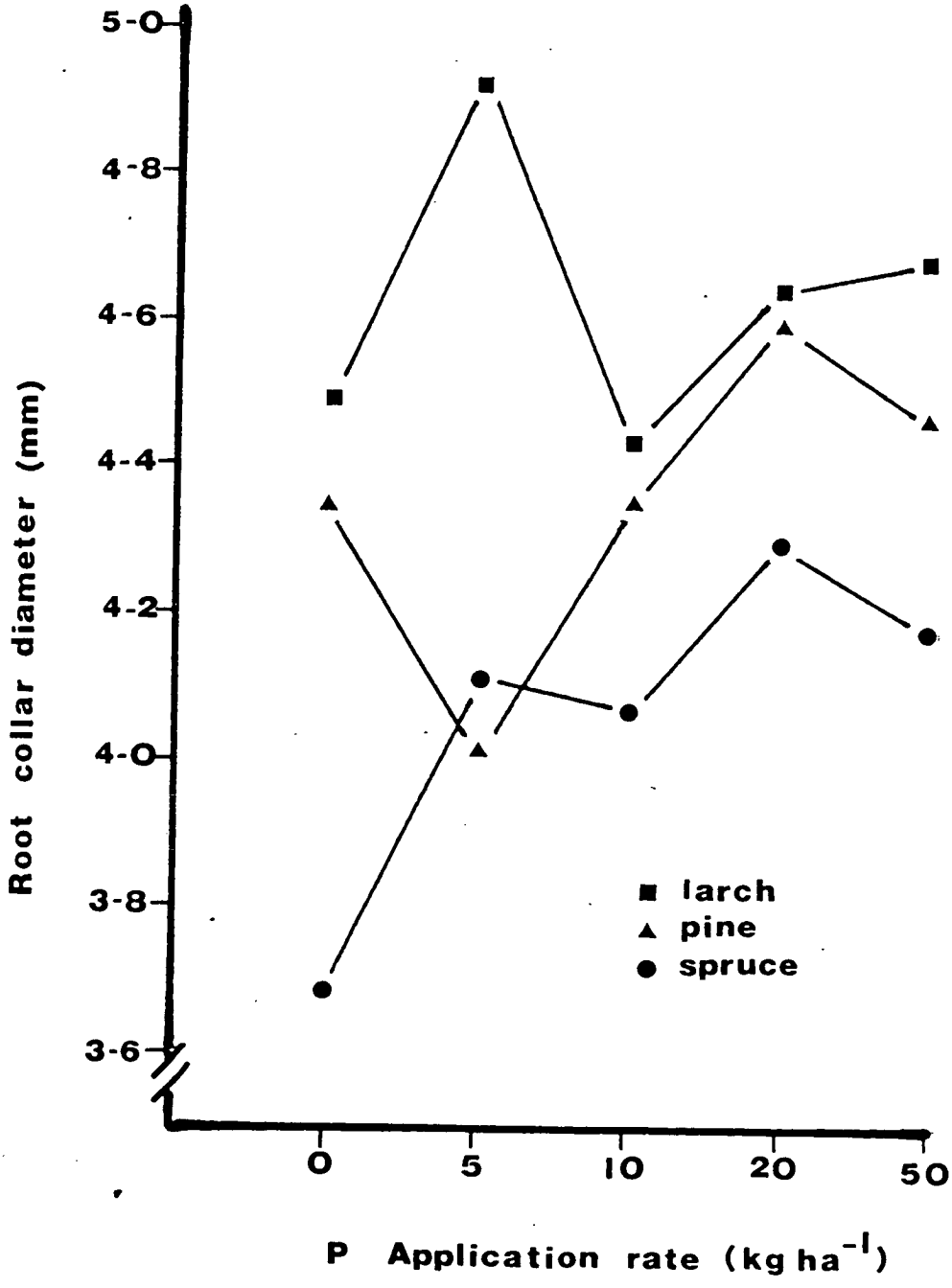


FIG. 3.1: Root collar diameter of species grown under different phosphorus fertiliser regimes. ($\bar{x}$, n = 10).

other treatments, which were all of a similar size.

ii) **Phosphorus bioassay**

The degree of sensitivity of the bioassay varied between the species (Fig. 3.2, Appendix 3B).

The only significant differences in ^{32}P-uptake by larch seedlings were between seedlings grown at the lowest rate of P application, P5, and the control and the P50 treatment. With pine, P0 and P5 plants took up more ^{32}P than plants from the P10, P20 and P50 treatments. Spruce seedlings showed the greatest sensitivity, P0 plants took up more ^{32}P than all the other treatments, likewise P5 plants. Again, P10, P20 and P50 treatments were similar.

iii) **Foliar analysis**

There were significant differences between the needle P content of each species, larch seedlings containing the highest amounts (Fig. 3.3, Appendix 3C) and pine the least. Needle P content of larch seedlings from the P50 treatment was higher than all the other treatments, and P20 plants contained more P than those from P0 and P5. Pine seedlings from both the P50 and P20 treatments had higher levels than all the other treatments. With spruce, although P50 was again higher than all the other treatments, the only other differences detected were between P0 and P10, and P0 and P20.

Although the concentration of the other elements, N, K, Ca and Mg in the needles varied between the species, there was no treatment effect on the foliar content of these elements (Fig. 3.4, Appendices 3D, 3E, 3F, 3G).

iv) **Root analysis**

There were significant differences between the P content of roots of each species, in this case, pine roots containing the highest amounts, and larch the least (Fig. 3.5, Appendix 3H).

There was no treatment effect on the root P content in larch seedlings, but in pine, P50 plants had higher root P levels than those from P0, P5 and P10 treatments, and P20 produced higher levels than P0. With spruce, the only differences detected

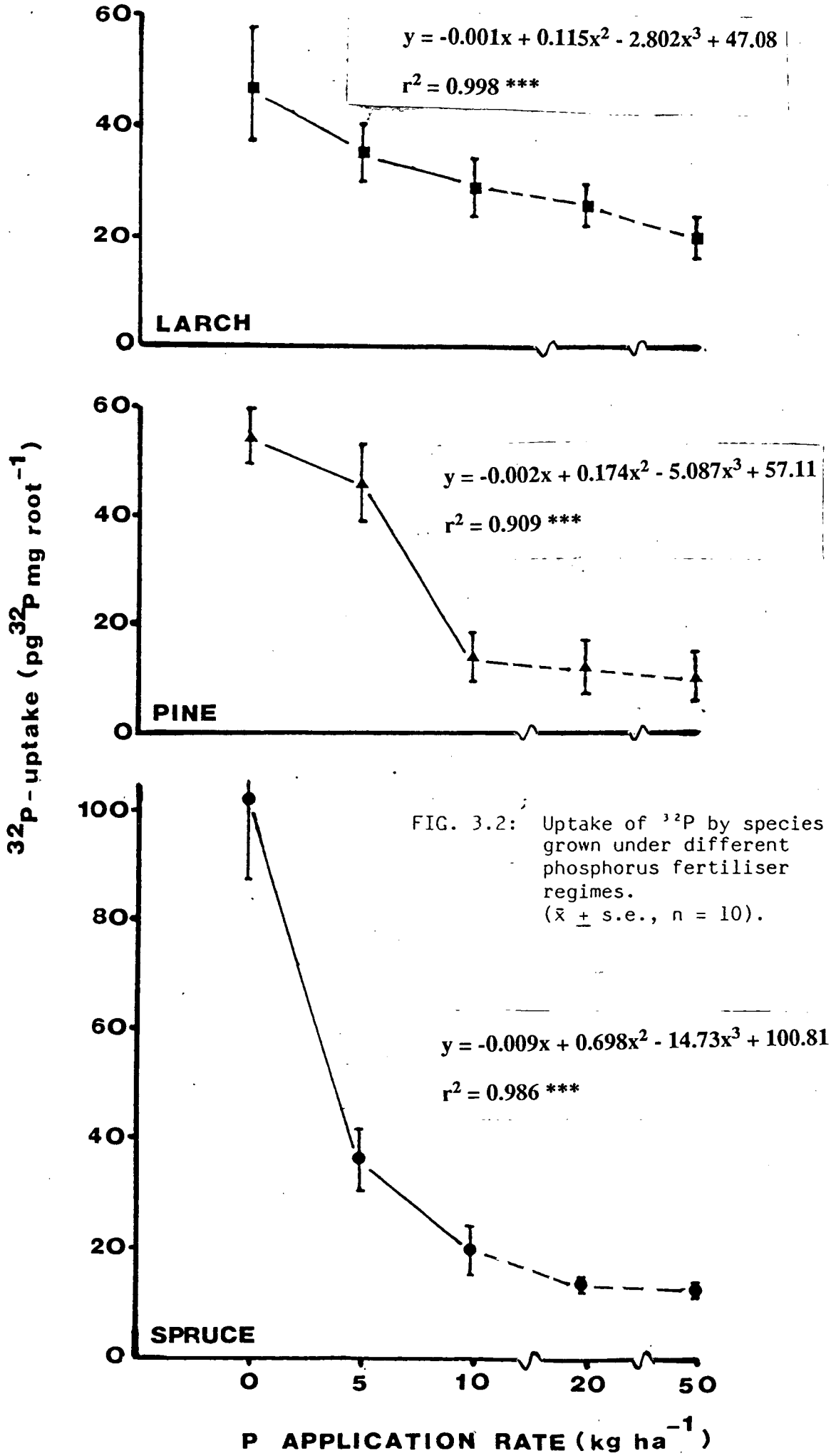


FIG. 3.2: Uptake of ^{32}P by species grown under different phosphorus fertiliser regimes.
($\bar{x} \pm$ s.e., n = 10).

■ $y = -0.001x^2 + 0.014x^3 + 0.255$, $r^2 = 0.982$ ***

▲ $y = 0.006x^3 + 0.108$, $r^2 = 0.977$ ***

● $y = -0.001x^2 + 0.02x^3 + 0.194$, $r^2 = 0.997$ ***

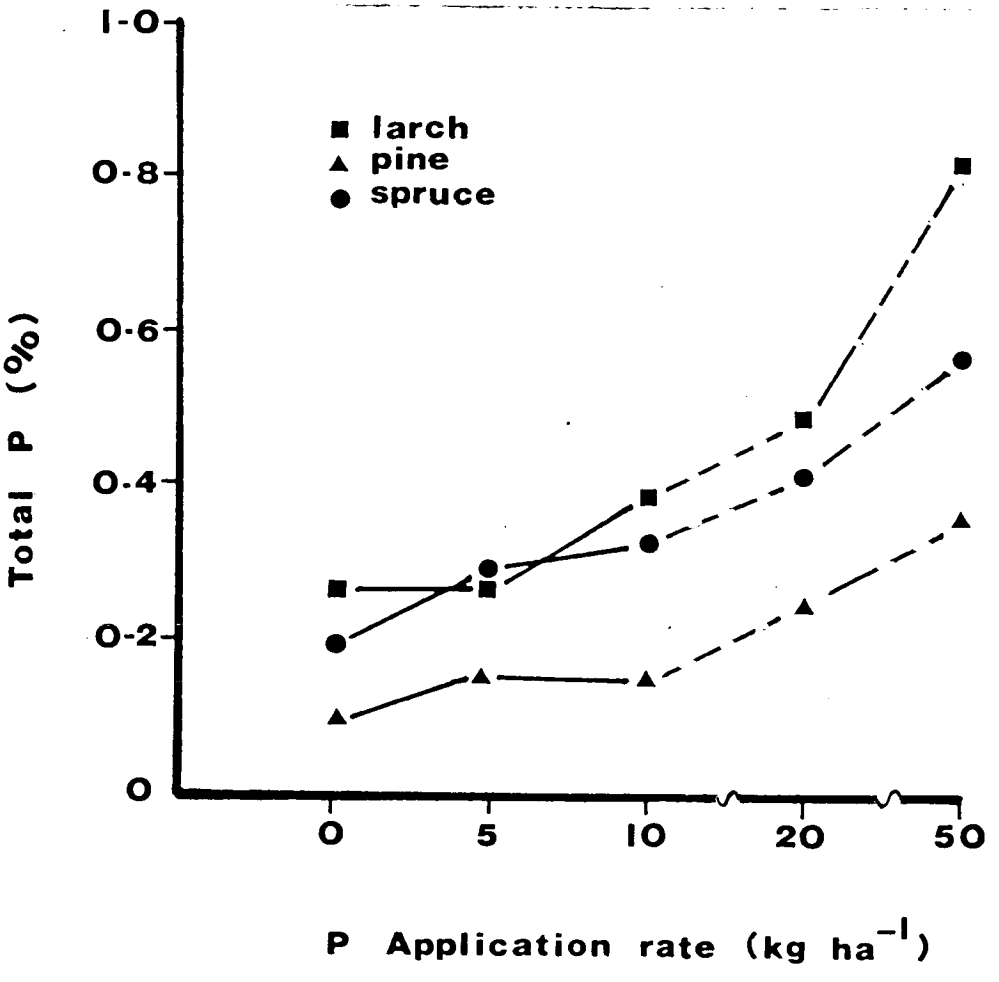


FIG. 3.3: Total P in needles of species grown under different phosphorus fertiliser regimes. ($\bar{x}$, n = 10).

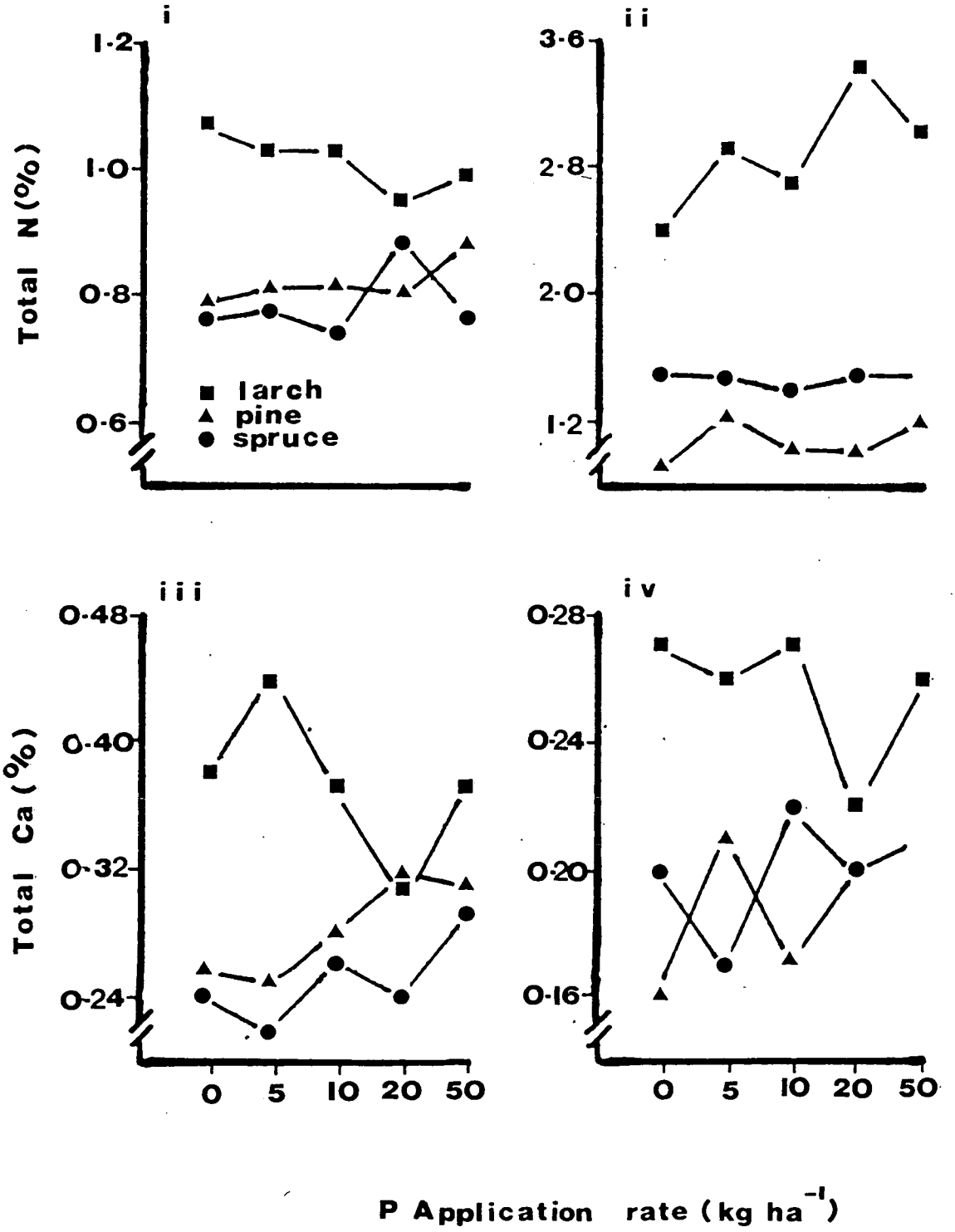


FIG. 3.4: Total (i) N (ii) K (iii) Ca (iv) Mg in needles of species grown under different phosphorus fertiliser regimes. ($\bar{x}$, n = 10).

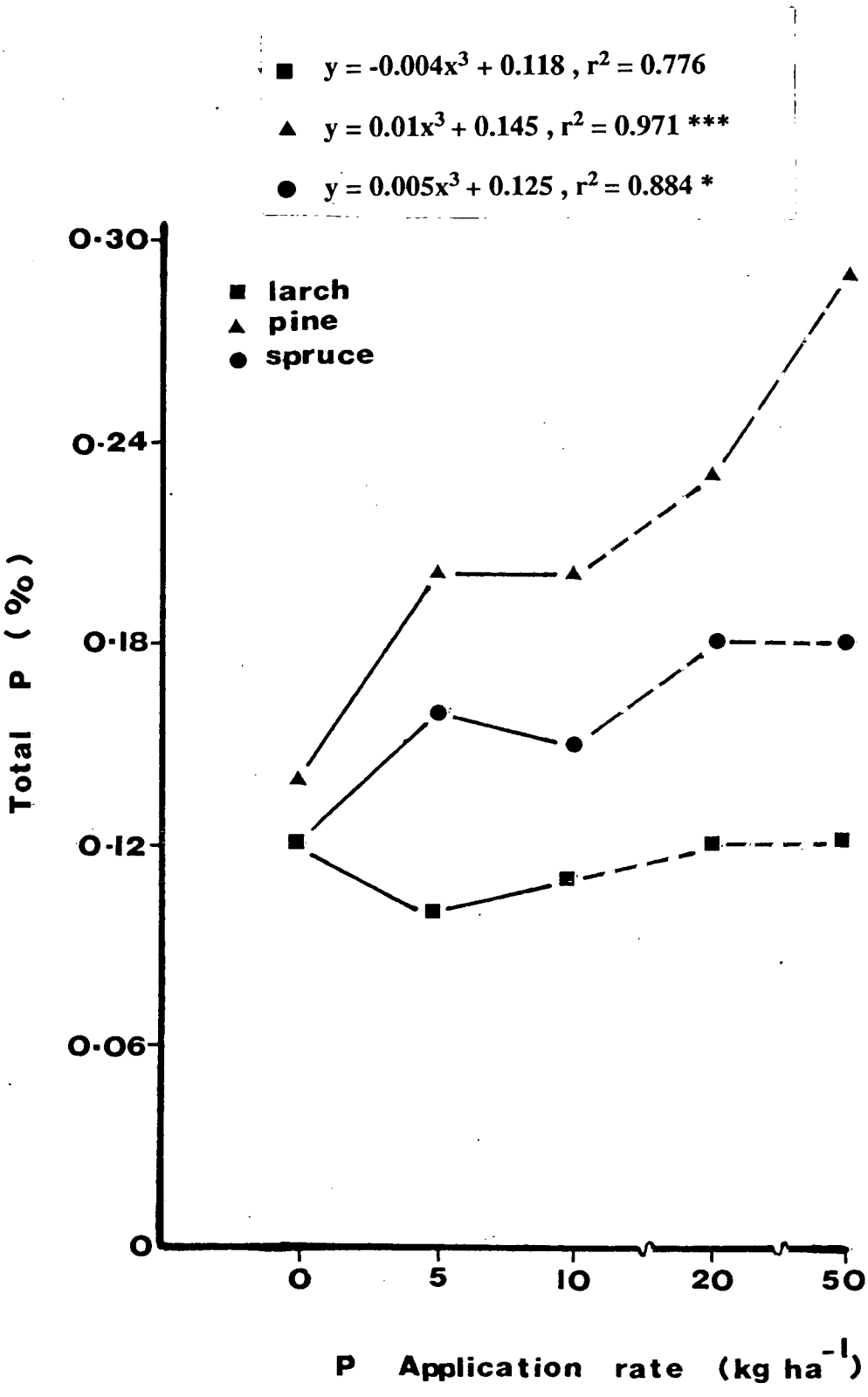


FIG. 3.5: Total P in roots of different species grown under different phosphorus fertiliser regimes.
($\bar{x}$, n = 10).

were between P50 and P0, and P20 and P0, the two highest treatments having higher root P concentrations than the control.

As found with foliar analysis, the concentration of N, K and Ca in the roots, although varying between species, was not affected by the different rates of phosphorus fertilisation (Fig. 3.6, Appendices 3I, 3J, 3K). The concentration of Mg in roots was not affected by the phosphorus treatment in larch or pine, but, with spruce, plants from the P20 treatment showed higher root Mg levels than those of the other lower treatments, P5 and P10 and the control (Fig. 3.6, Appendix 3L). However, the range of variation in Mg concentrations in spruce roots encountered is only 0.14 - 0.17 % with a 95 % confidence interval of 0.03 %, and so the results are barely significant and unlikely to be a treatment effect.

3.4 Discussion

The effect of different rates of phosphorus application on seedling productivity, as assessed by root collar diameter, is not marked. Although control spruce plants were smaller than treated plants, there were no significant differences in larch, and with pine, it was the P5 treatment which produced the smallest plants, and not the control. Over the course of a short-term experiment such as this, root collar diameter would therefore appear not to be a reliable indicator of the P-nutrition of seedlings.

The results of the bioassay held true to the original findings by Harrison and Helliwell (1979) in that, plants grown in a higher phosphorus regime took up less ^{32}P than those grown under the lowest phosphorus regime, for all three species. Differences in uptake were not detected between all treatments, the greatest significance being between the two lowest phosphorus applications and the higher treatments. This was, however, reflected in the foliar analysis of the seedlings - there is a positive correlation for all species between ^{32}P uptake and needle P content (Figure 3.7), as computed by SPSS. This would seem to indicate that the higher treatments provided sufficient P to meet the growth of predetermined buds at this size of plant, for one season. In fact, the phosphorus concentrations of the foliage for all the seedlings, except pine control plants, were above deficiency levels for 1 + 0

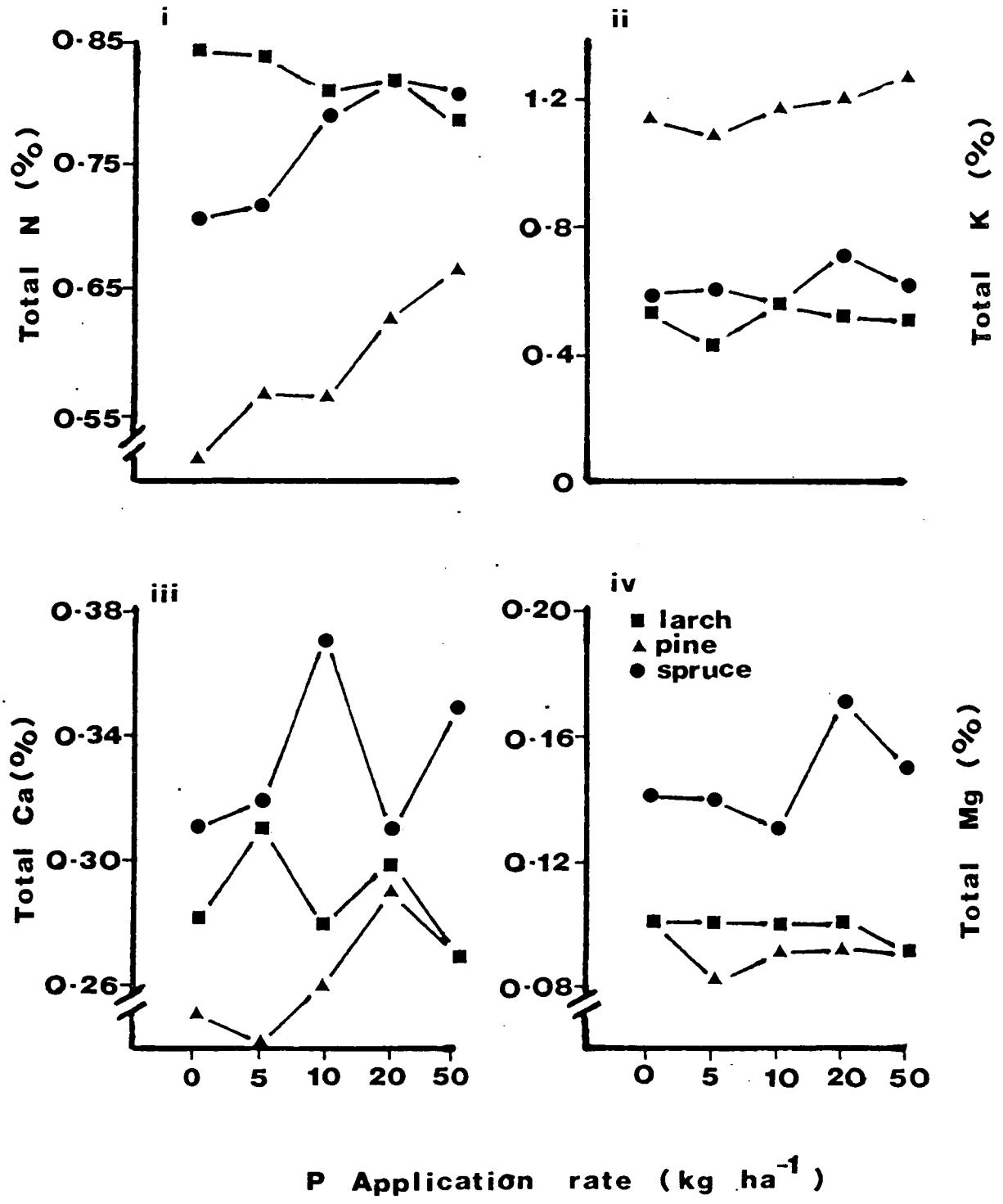


FIG. 3.6: Total (i) N (ii) K (iii) Ca (iv) Mg in roots of species grown under different phosphorus fertiliser regimes. ($\bar{x}$, n = 10).

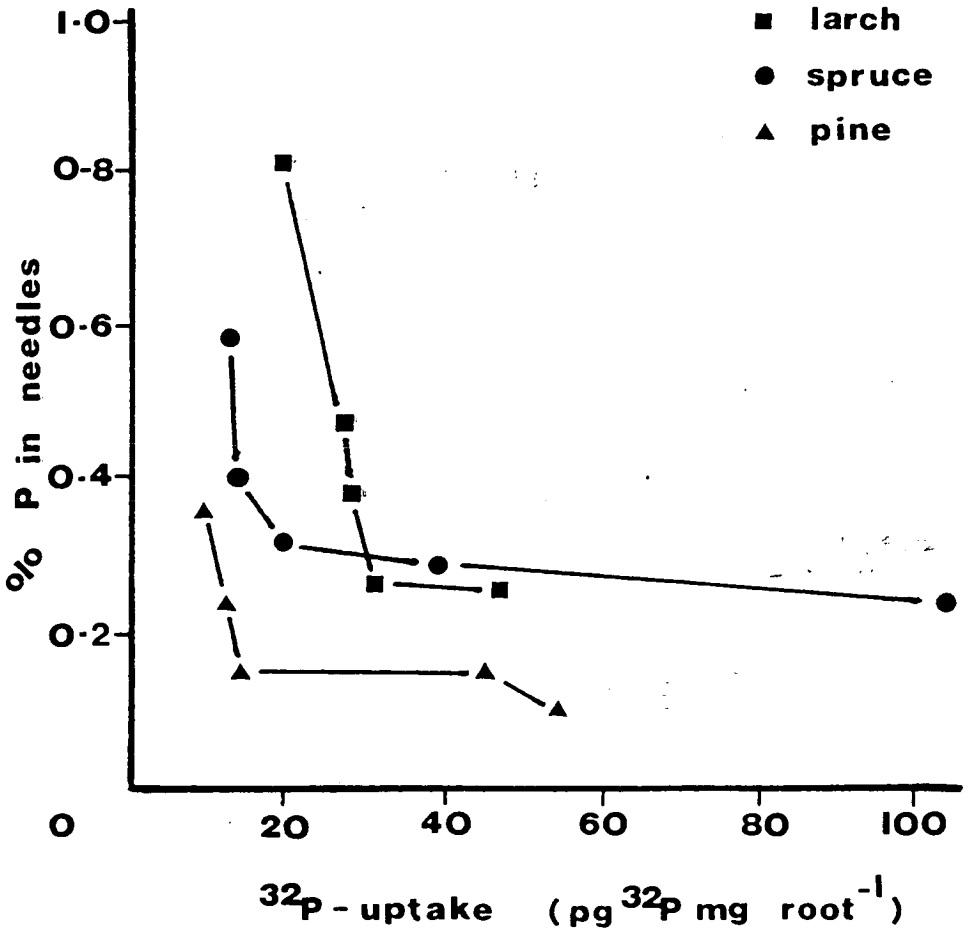


FIG. 3.7: Relationship of needle P content to ^{32}P-uptake.

seedlings sampled in autumn (Aldhous, 1975). Indeed, the higher treatments, particularly larch, showed levels either optimal or supra-optimal - to the extent that nitrogen deficiency had been induced in all plants, and they would have required N fertilisation had the experiment continued for another season.

Root analyses detected fewer differences in treatment means, none at all in larch, the species which showed the greatest treatment effect in needle P concentration. Conversely, pine roots showed the greatest treatment variation, while pine needles showed the least. Thus, although root P concentrations were consistently lower, and a less reliable indicator of overall plant P status, the fact that there were species differences between the distribution of P in the plant as affected by treatment, and the good correlation of ^{32}P uptake with needle P concentration, indicates that the bioassay is a good method of indicating overall plant P status.

CHAPTER 4

The Influence of Seasonal Variation on the ^{32}P Bioassay

4.1 INTRODUCTION

The interpretation of the results of a sampling programme requires that as many features of the collection as possible are standardised. This is because the interpretation is based on the comparison of the sample under test with known standards, thus standardisation of the time of collection of samples has been important in the diagnosis of nutrient deficiencies by foliar analysis. Considerable work has been carried out on the seasonal variation in foliar nutrient levels. In most crops, the least variation has been found in the early part of the dormant season (Everard, 1973). Touzet *et al.* (1969) showed that the variation in foliar phosphorus in Norway spruce, growing in central France, is least in the period from September to mid-November. In Britain, samples are normally collected in October.

The earlier examples of the use of the bioassay in the field with tree stands were all carried out in mid-summer - in July or August.

The bioassay is a supposed integrated index of the phosphorus status of the tree and the rooting environment, both of which are likely to vary with season. Seasonal variation is likely to be a consequence of temperature and moisture variations. The objective was therefore to firstly test the effects of seasonal differences on the results of the ^{32}P bioassay, in the field, with phosphorus and non-fertilised tree crops; to facilitate the investigation of the degree of phosphorus deficiency, and the sensitivity of the bioassay to different fertiliser regimes, with seasonality. Secondly, to attempt a separation of moisture and temperature effects by studying the responses of seedlings to variations in these when grown under controlled conditions in short term experiments.

4.2 THE INFLUENCE OF TEMPORAL VARIATION ON THE ^{32}P BIOASSAY

4.2.1 Site description

The experimental area is situated in the Myredykes Plantation of Wauchope Forest (Compartment 525) in the Roxburgh district of Borders region (National Grid Reference: NY 606984). The site is a very regular concave slope, generally gentle 4-8^{o}, becoming level at the bottom of the area, bounded by a small stream on either side. Elevation is 280 m a.s.l. with moderate exposure to the west, while the site receives 1300-1400 mm of precipitation per year. The soil is a surface water gley (Pyatt, 1970). This profile is typified by the shallow rooting depth as the virtually structureless Cg horizon is, in places, within 25 cm of the surface which results in a high water table for much of the year. In places there are very small pockets of peaty gley. The underlying geological formation is Tournaisian and Visean (Carboniferous Limestone Series). Prior to afforestation the area was open for grazing, the dominant vegetation being *Deschampsia caespitosa* along with *Juncus* (mostly *effusus*) and some *Molinia caerulea.*

Cultivation and drainage were carried out in 1961 by two runs with a shallow double mould board plough followed by a single run with a deeper single mouldboard plough (see Taylor, 1970). Ploughing was across the slope and this has tended to retain surface water, leading to instability in the tree stand.

Planting was carried out in 1962 with Sitka spruce (*Picea sitchensis* (Bong.) Carr.) origin Queen Charlotte Island.

Experiment Wauchope 17/83

An experiment established to investigate the interaction of thinning and fertilisation.

At the start of the experiment, the general yield class was 16. In 1983, a randomised block design of experiment was established with five replicates of six treatments. These were:

O - Control

N - Application of 200 kg/ha N as 440 kg/ha prilled urea

P - Application of 100 kg/ha P as 750 kg/ha unground phosphate rock

T - A low, selective thinning to remove one third of the standing basal area

TN - A combination of treatments T and N

TP - A combination of treatments N and P

The fertilisers were broadcast over the treatment plots in June 1983, after thinning in the late winter of 1982. Treatment plot size is 0.05 ha and each plot has on average, 180 trees and 100 trees in the unthinned and thinned treatments respectively. Dead and dying trees were removed from the unthinned treatments. The assessment plot size is 0.02 ha and contains, on average, 72 trees/plot.

During the winter of 1983/4, a heavy fall of wet snow resulted in considerable damage to thinned treatments in some blocks, which have since been abandoned from the experiment. Hence, in this study, only the unthinned plots were selected for sampling.

4.2.2 Methods

4.2.2.1 Root Sampling Procedure

Ten roots were collected from each of the replicates as described in Section 2.2.1, on the third Monday in every month from April 1984 for thirteen months to April 1985. In January 1985, however, sampling was not possible due to heavy snowfalls.

4.2.2.2 Phosphorus bioassay

The bioassay was carried out as described in section 2.2.2.

4.2.3 Results

The mean ^{32}P uptake values are presented in Figure 4.1 and Appendix 4A. Figure 4.1 also shows rainfall and temperature data, collected at the meteorological station at Kielder Castle, which is 4 miles from the experimental site.

Results were analysed by analyses of variance using the statistical computing package, GENSTAT. Prior to statistical analysis, all data were checked for normality and homogeneity of variance. The data for some months showed a degree of heterogeneity of variance. However, since it was desirable to make direct

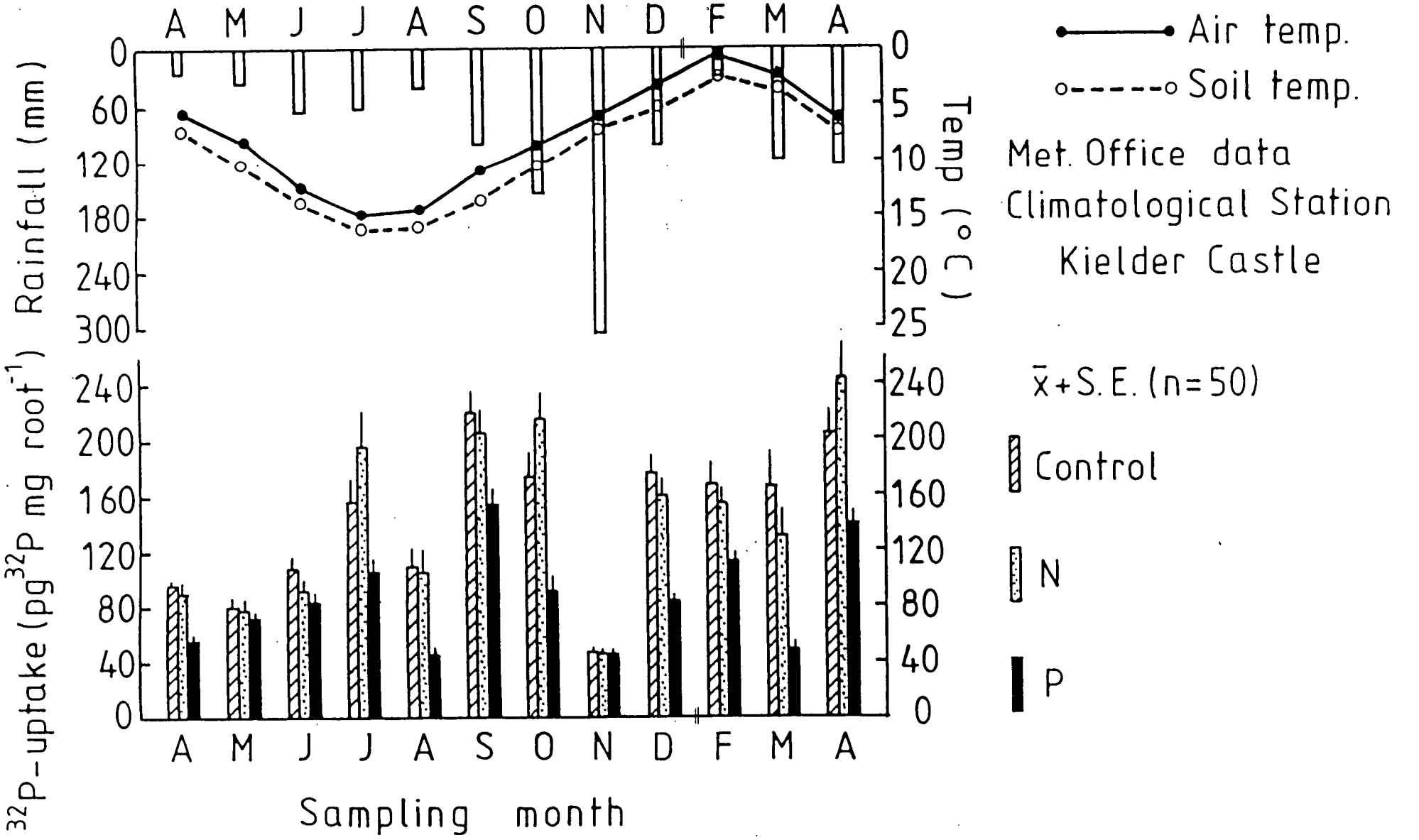


FIG. 4.1: Influence of month of sampling on ^{32}P-uptake and rainfall, air and soil (30 cm depth) temperatures over the sampling period.

comparisons between data sets, and in view of the fact that ANOVA is robust to departures from homogeneity of variance provided there are no missing values (Scheffe, 1959; Lindman, 1974) the data were not transformed. The ANOVA table is presented in Table 4.1.

Duncan's multiple range tests were used to establish significant differences between individual means.

The amounts of ^{32}P labelled phosphorus taken up by roots were least in the P plots throughout the year. They were significantly less in all months except for May, June and November. There were no differences in ^{32}P uptake by roots from the N and control plots throughout the year.

Roots from all treatments showed an increase in ^{32}P uptake over the spring, reaching a peak in July. Following low uptake levels in August, another peak was reached in September and October. Very low values were obtained in November, which also show the least treatment variation. Uptake was consistently high over the winter months, although rapidly increasing in April - to the extent that uptake in April 1985 was significantly greater than in April 1984.

There was no correlation between either rainfall or soil and air temperatures and uptake of ^{32}P labelled phosphorus. However, it was noted that the two months of lowest uptake in the P plots, August and November, corresponded to the periods of highest temperature and highest rainfall, respectively.

4.3 THE INFLUENCE OF TEMPERATURE ON THE ^{32}P BIOASSAY

4.3.1 Methods

4.3.1.1 Experimental design

Seedlings (1+0) of Queen Charlotte Island Sitka spruce and Skeena lodgepole pine were lifted from the Forestry Commission nursery at Tulliallan on 10th March, 1986. 27 seedlings of each species were selected for uniformity of form and vigour, and planted in University of California mix D2 compost (Appendix 4B), in large boiling tubes of 4 cm diameter on 11th March. The tubes were strapped into six wire baskets, with nine tubes in each (4 spruce and 5 pine or *vice versa*), and the plants

TABLE 4.1: ANOVA of Wauchope data

Source of Variation	df	SSQ	% SSQ	MSQ	VR
Treatment Block Stratum					
Treatment	2	1,191,878	6.0	595,939	3.88 *
P v Control	1	881,184	4.4	881,184	5.74 *
N v Control	1	310,695	1.6	310,695	2.02 ns
Residual	12	1,843,114	9.3	153,593	21.73 ***
Total	14	3,034,993	15.3	216,785	30.7
Treatment Block Root Time Stratum					
Time	11	3,822,773	19.3	347,525	49.2 ***
Treatment Time	22	601,449	3.0	27,339	3.9 ***
P v Control.dev	11	331,474	1.7	30,134	4.3 ***
N v Control.dev	11	269,975	1.4	24,543	3.5 ***
Residual	1752	12,381,671	62.4	7,067	
Total	1785	16,805,893	84.7	9,415	
GRAND TOTAL	1799	19,840,885	100.0		

kept in a heated greenhouse (20 °C day, 16 °C night), well watered and illuminated, although without artificial lighting, until 16th April, 1986.

On 16th April, two baskets were placed in each of three water baths, so that the level of water was higher than the compost in the tubes. The baths were maintained at temperatures of 5, 12 and 20 °C by Grant heating units with stirrers and Grant cooler units for the two cooler temperatures. The water surfaces were covered with pieces of expanded polystyrene to act as insulation (Plate 4.1). These systems proved effective to ± 0.5 °C when checked at regular intervals in the two days before starting the experiment.

The seedlings were left in the baths, adequately watered until harvesting on 16th May 1986.

4.3.1.2 Root sampling procedure

On 16th May, the tubes were removed from the baskets. The seedlings were carefully removed, with the compost, from the tubes. Loose compost was shaken off, the seedlings appropriately labelled and their root systems washed under running tap water.

4.3.1.3 Phosphorus bioassay

The seedlings were assayed immediately after washing as described in section 2.2.2.

4.3.2 Results

The results were analysed by one-way analysis of variance using the statistical computing package, SPSS. Prior to statistical analysis, all data were checked for normality and homogeneity of variances.

No difference was found in the uptake of ^{32}P, for either species, attributable to the differential root temperature environments (Figure 4.2, Appendix 4B). There was also no difference between the behaviour of the two species.

4.4 THE INFLUENCE OF MOISTURE ON THE ^{32}P BIOASSAY

4.4.1 Methods

4.4.1.1 Experimental design

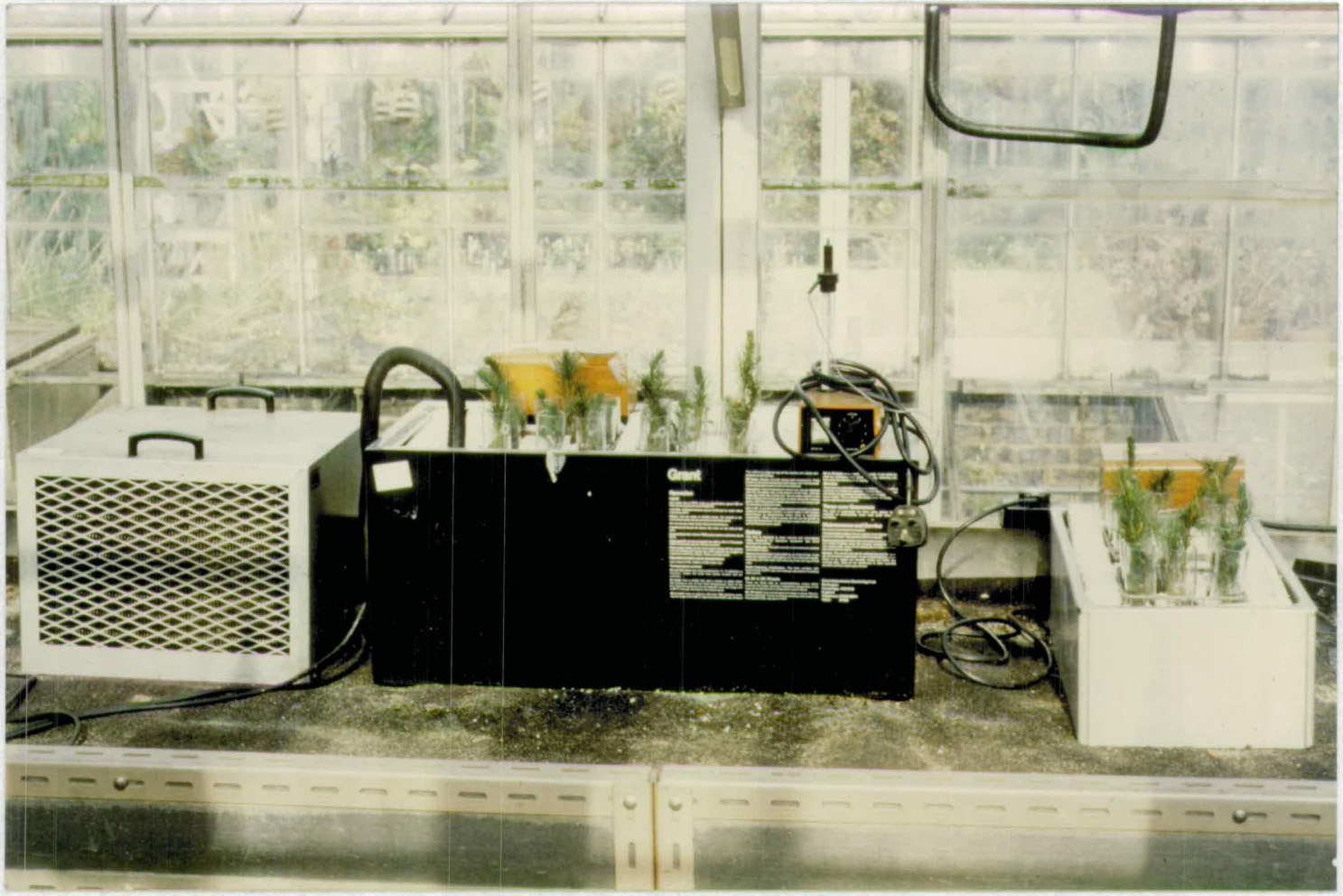


PLATE 4.1: Experiment 4.3 Set-up.
The Influence of Temperature on the ^{32}P Bioassay.

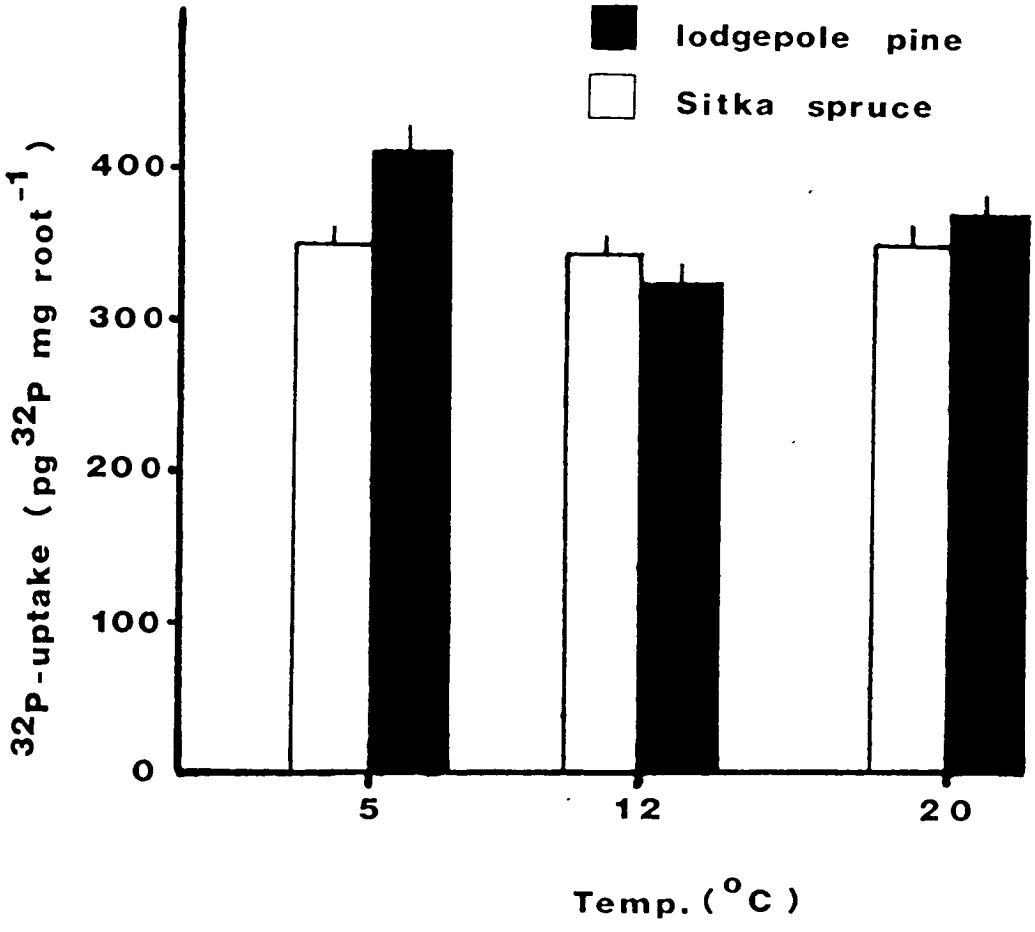


FIG. 4.2: Influence of temperature on ^{32}P-uptake. (pre-bioassay)
($\bar{x}$ + s.e., n = 9).

1+0 seedlings of Queen Charlotte Island Sitka spruce and Skeena lodgepole pine were lifted from the Tulliallan nursery of the Forestry Commission on the 10th March 1986. Seedlings of each species, selected for uniformity of form and vigour, were planted in 8 cm square pots on 11th March in University of California mix D2 compost. The plants were kept well illuminated although without artificial lighting in a heated greenhouse (20 ^{o}C day, 16 ^{o}C night) and watered to, or near, field capacity, until 17th April 1986.

On the 17th April, each pot was numbered, weighed and the pot covered with polythene film to prevent evaporative water loss. The plants were then laid out in ten blocks of nine plants of each species (Plate 4.2).

Subsequently, randomly selected pots were weighed daily, and when the soil reached a predetermined estimated pF value (Section 4.4.1.2), one plant of each species was removed from a block, its xylem pressure potential measured, (Section 4.4.1.3), the plant harvested, and the bioassay carried out (Sections 4.4.1.4 and 4.4.1.5).

4.4.1.2 Measurement of pF

Prior to covering the tops of the pots, samples of the compost were taken and oven-dried, to a constant weight, at 85 ^{o}C, to determine percentage moisture content. The pF value of the soil corresponding to this was estimated from Table 4.2.

Table 4.2: Percentage moisture contents of a 50:50 fine sand/peat mix (oven dry basis) at different pF values (Boggie, 1970).

%.m.c.	69.0	65.7	36.0	20.0	14.8	9.6	5.8	4.7
pF	**0.4**	**1.0**	**1.5**	**2.0**	**2.3**	**2.7**	**3.4**	**4.2**

On every subsequent day, randomly selected pots were weighed to determine transpiration water loss and the new moisture status of the compost estimated. The percentage moisture content was then related to the pF value given by Boggie (1970). It was decided to take sample trees at pF values of 1.5, 1.8, 2.0, 2.4, 2.7, 3.0, 3.4

PLATE 4.2: Experiment 4.4 Set-up.
The Influence of Moisture on the ^{32}P Bioassay.

and 4.2. The pF value of the compost at the start of the experiment was 1.4. Boggie (1970) reported that most of the water available for plant growth is removed from this compost, which is 50 % sand/50 % peat by pF 2.7 - 3.4, although water can be absorbed by plants up to pF 4.2.

Control plants were also designated which were weighed daily and water added, if necessary, to adjust the total pot weight to that at the beginning of the experiment.

4.4.1.3 Measurement of xylem pressure potential

The terminal shoot of the seedlings taken for sampling was removed with a sharp razor blade, and inserted in a pressure chamber. Pressure was exerted on the shoot by use of compressed nitrogen until xylem sap appeared at the cut surface of the xylem. The end point required is the balancing pressure just required to hold the xylem sap at the cut surface. The pressure applied is then equal to the xylem pressure potential in the shoot at the time of cutting (Ritchie and Hinckley, 1975).

The time of cutting was important due to diurnal variations in the rate of transpiration, and so measurements were always made at 10 a.m. on the day of sampling. This time was selected for practical convenience, and was probably not the time when the plants would be most stressed.

4.4.1.4 Root Sampling procedure

One plant of each species from each block, giving ten replicates per treatment for both species, was sampled at the appropriate times together with four control plants for each species.

The plants were carefully removed, with the compost, from the pots. Loose compost was shaken off, the seedlings labelled and their root systems washed under running tap water.

4.4.1.5 Phosphorus bioassay

The seedlings were assayed immediately after washing as described in Section 2.2.2.

4.4.2 Results

4.4.2.1 pF values

Initial transpiration rates, between April 17 and May 1 were around 5 g moisture per day, reducing to about 3 g per day until May 29 (Figure 4.3). From May 29 until June 6, when the soil reached a pF of approximately 3.0, transpiration water loss was only 1 g per day. After June 6, fine root death started and the experiment was terminated.

4.4.2.2 Xylem pressure potential

The shoot xylem pressure potential of the control plants remained constant throughout the experiment at 0.45 $\pm$ 0.05 MPa. The treated plants showed a gradual increase in potential up to a maximum of 0.12 $\pm$ 0.01 MPa at pF 3.0 (Figure 4.3).

4.4.2.3 Phosphorus bioassay

The results are expressed as the mean difference in uptake between the controls and the treated plants (Figure 4.3, Appendix 5C).

They were analysed by one-way analysis of variance using the statistical computing package, SPSS. Prior to statistical analysis, all data were checked for normality and homogeneity of variances.

If an increase in ^{32}P-uptake by the treated plants over that of the controls is regarded as an increase in phosphorus stress, then prolonged drought is seen to induce such a stress.

For both species, when the pF was less than 2.0, ^{32}P uptake by treated and control plants was similar. At pF values greater than 2.0, treated plants took up more ^{32}P than control plants. However, there were no differences between treatments at any of the pF values recorded above 2.0, except that spruce seedlings took up more ^{32}P at a pF value of 2.7 than the seedlings sampled at pF 2.0 (Figure 4.3).

4.5 DISCUSSION

The measurements of seasonal pattern of ^{32}P uptake in the field samples indicated that there were differential degrees of phosphorus stress over the year. These could be the result of variations in either temperature or soil moisture stress.

The temperature in the previous rooting environment had no significant effect on the results of the bioassay. This is not what might have been expected. Uptake of

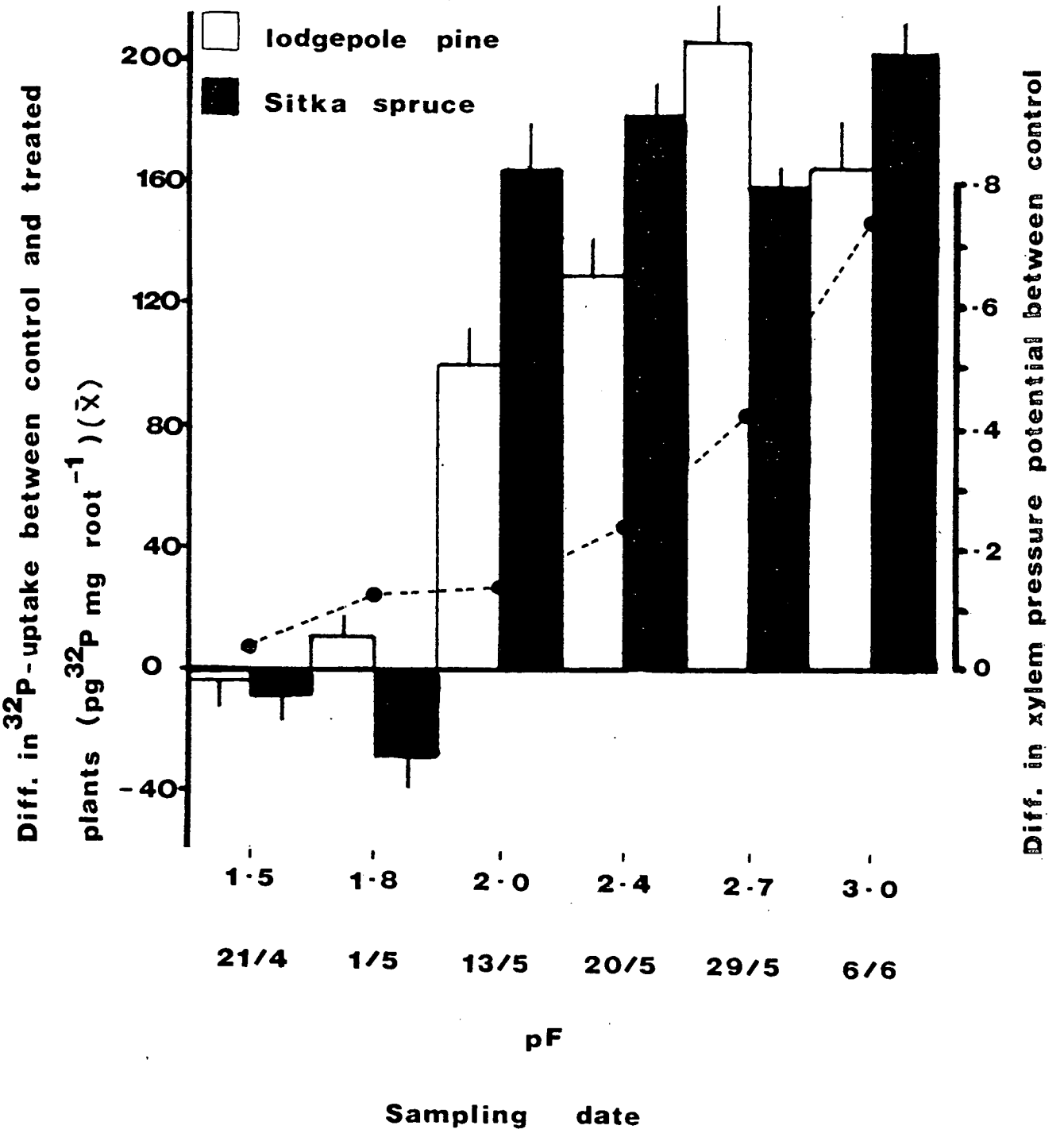


FIG. 4.3: Influence of moisture status on ^{32}P-uptake and xylem pressure potential. ($\bar{x}$ + s.e., n = 10).

phosphorus by roots has been shown to be an active process and therefore a function of the metabolic activity of the plant. The effect of temperature on metabolic rate is well established, generally being seen to follow a positive relationship where Q10=2. Jensen and Petterson (1980) noted that the uptake of N, P and K was approximately doubled in Scots pine seedlings, as the temperature was increased by 10 ^{o}C in the physiologically interesting range 5-25 ^{o}C. Simpson (1960, 1965) found, in a pot experiment with cereal, that a temperature increase of 5 ^{o}C of the rooting environment increased phosphorus uptake without applying phosphorus fertiliser.

The lack of response by the roots to temperature may be attributed to several factors. The preconditioning may have been insufficiently prolonged to induce phosphorus stress on the plants grown at lower temperatures, or even to alter their metabolic rates, and when the roots were placed in the bioassay solution which was maintained at 18 ^{o}C, their uptake patterns proved to be similar.

This, however, was not reflected in the field study, where differences in phosphorus stress were detected over a similar time period.

Bowen (1970) found that the uptake of phosphorus by roots grown at lower temperatures was sustained along their length. In contrast, uptake by roots from higher temperatures was greatest in the apical centimetre and decreased sharply along the roots. The sustained uptake may have been sufficient to compensate for a lower rate of uptake.

Alternatively, Neilsen *et al.* (1960) found that the concentration of ions was lower in roots grown at 5 ^{o}C than those at 10 ^{o}C in Lucerne. This may be because ion transport from the roots to the rest of the plant, being an energy-dependent process, is impeded at 5 ^{o}C. A higher root phosphate content would reduce the amount of ^{32}P taken up during the bioassay.

Similarly, water is important in any energy-dependent process and its shortage will impede the uptake and transport of phosphate ions by the root. Its role in these processes is also a physical one, in that the ions are taken up and transported in the plant in solution and furthermore arrive at the root surfaces in the soil by diffusion in

solution. The increase in phosphorus stress with decreasing water availability, shown by the bioassay results in the second experiment, could be a consequence of both these processes. Changes in water viscosity at lower temperatures may also have impeded uptake in the first experiment.

Although phosphorus stress was induced in the treated plants one month after the start of droughting, further increase in water stress, as indicated by the pF in the soil and the shoot water potential, did not necessarily mean a further increase in phosphorus stress. This 'cut-off' would suggest that, in this case, the mechanism inducing the phosphorus stress is a physical one, limiting the amount of solution in the soil which can be drawn on by the plant. This is instead of a gradual slowing of the energetic reactions in the plant because of a lack of moisture.

It would appear then, that the direct effect of neither temperature nor moisture provides a satisfactory explanation of the seasonal effect found in the field study.

This is perhaps not surprising as the degree of availability of phosphorus to the plant is an integration of many factors, namely the total rooted soil volume, root density, the concentration of phosphorus in the soil solution and the rate of release from unstable organic and inorganic pools in the soil (Mohren *et al*, 1986) and also the demand of the tree. All these factors, and the interactions between them, are susceptible to seasonal variability.

Uptake of nutrients from the soil is largely governed by rooting intensity and the extent to which mycorrhizal fungi supplement this (Bowen, 1984). The rooting intensity and distribution pattern is a reflection of edaphic and climatic factors (Deans, 1979; Ford & Deans, 1977). Deans (1979) found that the biomass of fine roots in a 14-year-old Sitka spruce plantation fluctuated with maxima in late May and mid July. The earlier peak coincides with increasing soil temperatures during a period of high incident precipitation and the latter developed when the soil was re-wetted. This was was shown by Roberts (1976) in a Scots pine plantation who found that root growth increased with increasing temperatures in spring to reach a peak in April and May, but, despite further temperature increases into June and July, root activity declined after

May as the soil water content declined.

This phenological pattern is reflected, to some extent, by microbial activity and litter decomposition and hence nutrient availability. Alexander (1977) states that microbial activity is highest in the spring when soil temperature rises to an optimum level, decreases in summer due to low moisture and increases again in the autumn when the soil is rewetted. Gupta and Rorison (1975) and Veresoglou and Fitter (1984) found the peak availability of phosphorus in May and June, and Harrison (1979) found phosphatase activity, which mediates the release of inorganic phosphate from organically-bound phosphorus, to be highest in summer.

Nutrient release by microbiological decomposition will be negligible in the controlled experiments, where the plants were grown in a sand/peat mixture which is relatively sterile and chemically inert (Boggie, 1970).

The transport of ions to the root is influenced by similar factors. McColl (1973) shows that the transport process is controlled by three environmental factors - soil temperature, the duration of the dry period before moisture flow and the total amount of moisture flow during the movement of wetting fronts.

These phenomena are reflected in the results of the field study. All treatment plots show a gradual increase in spring and early summer, up to July. During this period, increasing rainfall and temperature renders more phosphorus available, but this, however, is matched by an increased demand by the trees for phosphorus to satisfy the requirements of fine root growth and shoot elongation (Owens and Molder, 1976; Ford and Deans, 1977). Consequently, by late July, when shoot elongation is completed, the level of requirement drops as reflected in the August sample. However, depletion of the available phosphorus pool during the early summer months, combined with relatively low rainfall in August and ongoing leaf initiation in the tree, results in a high degree of stress during September and October. After this time, the gradual rewetting of the soil profile, maximum precipitation falling during November, and the progressive dormancy of the buds in November means that there is a very low degree of stress at this time. Falling temperatures and less rainfall in

December again increases stress and the continuing low temperatures and rainfall maintain a constant level of P demand during the winter months. Malcolm and Cuttle (1983) and Titus (1985) all recorded winter peaks in P release in drainage, corresponding with reduced uptake during these months, but also explicable in terms of microbial death. Although most P in the soil is present as abiotic organic P, considerable amounts can be immobilised by soil fauna and micro-organisms (Stewart and McKercher, 1982) and release to the soil solution depends upon the lysing of cells after the death of micro-organisms due to heating and drying of the soil (Cole *et al.*, 1977) and freeze/thaw cycles (Cole *et al.*, 1977; Chapman *et al.*, 1978). The low temperatures of the winter months, and the heavy snowfalls which prevented sampling in January, may have had a sterilising effect on the soil. With the increase in temperature in the spring, decomposition of organic phosphorus is enhanced as a result (Gupta and Rorison, 1975), leading to a decrease in the degree of stress in March.

The increased demand in April 1985 from April 1984 may be a consequence of the severity of the preceding winter, and the clemency of the spring. The winter of 1983/4 was considerably milder than 1984/5 and there may have been a lesser loss of P in drainage. The higher rainfall in April 1985 will also have encouraged metabolic activity and the earlier onset of shoot activity, thus increasing the demand. However, the onset of shoot activity is also related to winter chill and accumulated warmth subsequently, the extent of which is not quantified for the previous year.

The effect of treatment is marked in that roots from the P plots consistently took up less ^{32}P than those from the other treatment plots. This effect was most marked following those conditions, already described, enhancing the availability of phosphorus. The effects of nitrogen fertilisation did not have any significant effect on the phosphorus status of the trees, compared with control plots, throughout the year. This is in contradiction with some workers (e.g. Miller & Williams, 1969; Taber & McFee, 1972) who found that nitrogen applications enhanced phosphorus uptake in *Pinus contorta* Dougl. and *Pinus radiata* D. Don. respectively. Maftoun and Pritchett

(1970) and Mohren *et al.* (1986) however, found that nitrogen application suppressed phosphorus uptake in *Pinus elliottii* Engelm. and *Pseudotsuga menziesii* (Mirb) Franco. Ingestad (1971) states that, ideally, a nutrient supply should contain macronutrients in the same proportion of the optimal nutrient content of the species. In this case, the trees showed no obvious nutrient deficiency symptoms in any of the treatment plots, and the relatively low levels of ^{32}P uptake indicate that, for all treatments, the trees were not very stressed for phosphorus, although differences in their phosphorus status were detected. Thus, it would seem that, the fertiliser applications did not significantly imbalance the N/P ratio in the trees.

These studies indicate that the bioassay provides an integrated index of the phosphorus available to the tree, which it is capable of utilising, and the demand by the tree-parameters susceptible to considerable variation throughout the year. If the bioassay is to be used as a predictor of the phosphorus requirement of trees, then this variability must be minimised to provide the standards required for comparison with samples under test.

In the season studied the optimal time for sampling would be during late September or October. This period precedes winter freezing and follows summer drought, and sampling at this time therefore reduces variability due to climatic extremes, or increased demands as a result of physiological processes.

This would be the situation in most years, although sampling would require adjustment if seasons of particularly extreme climatic conditions were encountered.

CHAPTER 5

The Influence of Spatial Variation on the ^{32}P Bioassay

5.1 INTRODUCTION

Temporal variation has been considered, (Chapter 4), but of equal importance is spatial variation.

High spatial variability of the chemical and physical properties of the forest floor (e.g. Quesnel and Lavkulich, 1980) and of forest soils in general (e.g. McFee and Stone, 1965; Lowe, 1972; Blyth and MacLeod, 1978) is well known, most workers agreeing that the soil component of a forest floor cannot be properly characterised unless numerous samples are collected. Blyth and MacLeod (1978) concluded that the relationship between tree growth and soil nutrient status may not be detectable because of short-range spatial variability, while Ball and Williams (1968) found that, even in a non-cultivated, freely draining brown earth which is generally regarded as a uniform soil, spatial variability in a circle of one metre radius prevented detection of seasonal trends in soil chemical properties.

This problem is exacerbated by pre-planting cultivation practiced in the establishment of plantation forests. Single mouldboard ploughing deposits ribbons of peat between adjacent furrows, creating a forest floor with three physical features - a furrow, flat and ridge (Figure 5.1). The young trees are planted on the upturned ridges.

In addition to topographical differences of the ploughing positions, different soil horizons are associated with each feature. The furrows are characterised by litter overlying mineral soil, while the flats have litter and fermenting layers overlying organic soil and the ridges are elevated by the peat removed from the furrows (Ford and Deans, 1977).

This obviously has implications for nutrient availability. Carlyle (1984) found in an unfertilised Sitka spruce stand, that nitrogen mineralisation was greater on the flat

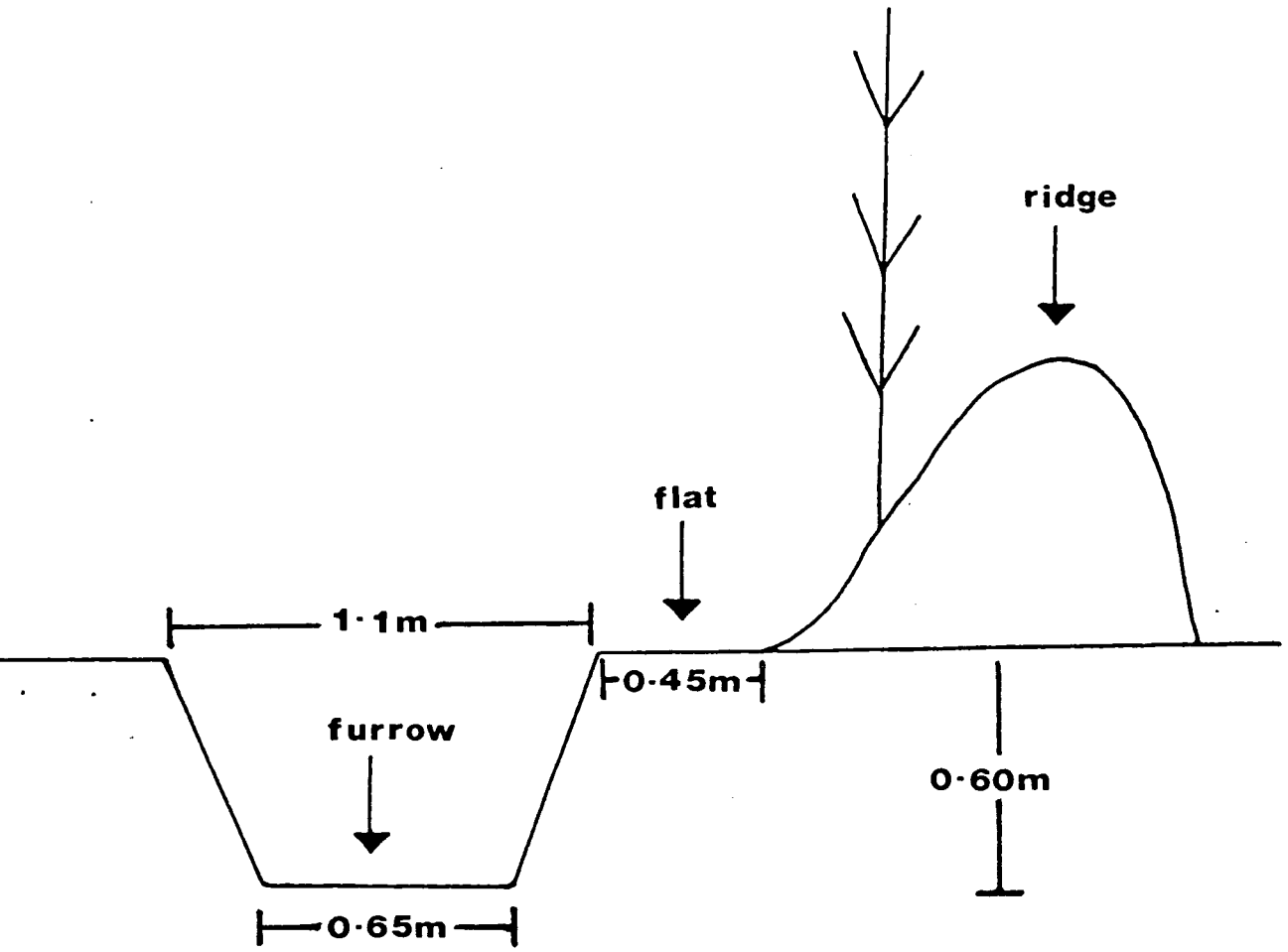


FIG. 5.1: Diagrammatic section across ploughing to indicate sampling positions (S60/-/t ploughing). (Taylor, 1970).

than the ridge, which he attributed to the higher moisture content of the flat position. Ford and Deans (1977) found the presence of both macronutrients, and fine roots, to be concentrated close to the surface of the ridges in response to the lower soil moisture tension there, especially around the tree base, because of stemflow inputs. The implication for the bioassay would appear to be that roots collected from the ridge might take up less ^{32}P than those from the flat or furrow. If positional differences exist, then standard sampling may have to adopt the flat sampling position which is less prone to moisture fluctuation.

In Sitka spruce seedling culture, Coutts and Philipson (1976) found roots well supplied with phosphorus showed stimulated growth. However, translocation of nutrients occurred to roots poorly supplied with phosphorus. Thus, spatial variability for the purposes of the bioassay may be disguised.

To examine potential spatial variability in the uptake of P, a sampling programme was devised in Sitka spruce and lodgepole pine stands established on deep peat. Samples were taken in different seasons from the three microtopographical positions of the ploughed site (Figure 5.1). Physical and chemical properties of the forest floor were also determined.

To investigate within plant variability a split root pot experiment was established using Sitka spruce and lodgepole pine. Either side of the root system was then differentially supplied with P.

5.2 SPATIAL VARIABILITY OF THE FOREST FLOOR

5.2.1 Site description

The investigation of spatial variability was carried out in a Forestry Commission experiment designed to demonstrate nutrient deficiency symptoms on lodgepole pine and Sitka spruce on deep acid peat.

Experiment Eddleston 7

The experiment is situated in Eddleston Forest (compartment 20) near Leadburn, fifteen miles south of Edinburgh (O.S. Grid Ref. NT 234 540). The site is a uniformly flat peat area, of open aspect, being moderately exposed to South-West and

Westerly winds, but slightly sheltered from the East wind by higher ground. Elevation is 282 m a.s.l. and the site receives 850 mm rainfall per annum. The soil is a deep peat, of seven metres depth overlying boulder clay.

Prior to afforestation, the vegetation was dominated by *Calluna vulgaris*, with frequent *Erica tetralix*, *Trichophorum caespitosum*, *Eriophorum vaginatum* and an abundant *Sphagnum* moss layer.

Planting was carried out in 1967, following S60 ploughing at 1.8 m spacing. Each plot consisted of 88 trees in a rectangular arrangement of 11 x 8 trees, the innermost 30 trees constituting the assessment area. The species used were Sitka spruce (*Picea sitchensis* (Bong.) Carr.), Queen Charlotte Island provenance, and coastal and inland provenances of lodgepole pine (*Pinus contorta* Doug.), from Long Beach, Washington and Prince George, British Columbia. The fertiliser treatments imposed at planting, seven in all were;

+PKCa - P, K and Ca applied

-Mg - N, P, K and Ca

-O - N, P, K, Ca and Mg

-K - N, P, Ca and Mg

-P - N, K, Ca and Mg

-N - P, K, Ca and Mg

-ALL - no fertilisers applied

These treatments were designed deliberately to create individual nutrient stresses. The layout of the plots and the fertiliser regimes are given in Appendix 5A.

5.2.2 Methods

5.2.2.1 Root sampling procedure

Ten roots were collected from each of the furrow, ridge and flat (Figure 5.1) as described in Section 2.2.1. The plots selected for sampling were the +PKCa plots (nos. 1 and 32, Appendix 5A), the standard treatment for such a site, and the -P plots (nos. 5 and 28, Appendix 5A). The rates of fertiliser applied to these plots are given in Appendix 5B. The inland provenance of lodgepole pine was sampled.

Sampling was carried out in the first weeks of May, July and September of 1984 and February 1985.

5.2.2.2 Phosphorus bioassay

The bioassay was carried out as described in Section 2.2.2.

5.2.2.3 Soil Sampling and Analysis

On July 27th, 1985, soil samples were taken from 6 randomly selected points on the ridge, flat and furrow positions respectively in each of the four treatments. Samples were collected to a depth of 9 cm using a 6.6 cm internal diameter corer; this was judged to be the approximate depth from which root samples are collected for bioassay. Each core was then transported to the laboratory in a sealed polythene bag and then weighed before and after air-drying in an air-circulation oven at 35 ^{o}C. Air drying may affect several soil properties (Allen *et al.*, 1974) but was justified on practical grounds and because relative values between the respective topographic positions were desired.

The air-dry material was passed through a 2 mm mesh sieve. The soil passing through this size of mesh contains almost the whole of the nutritionally important fraction in the soil (Allen *et al.*, 1974). The soils were then ground in a hammer mill and the following properties determined.

(i) pH in Water

A sample of soil was placed in a 50 ml beaker and covered with distilled water in the ratio 2.5 water:1 soil. Water was preferred to $CaCl_2$ in order to mimic the field situation as much as possible. This was stirred frequently over a 2 hour period. The electrode of a Kent digital pH meter was immersed in the slurry and the pH recorded to one decimal place.

(ii) Moisture content

The moisture content of the air-dried soils was estimated by the difference between the fresh weight and the air-dried weight of the soil cores. Air-dry sub-samples, approximately 1 g weight were dried to constant weight at 105 ^{o}C. After cooling in a desiccator the samples were re-weighed and total moisture content

expressed as percentage of oven dry weight.

(iii) Bulk density

Bulk density was calculated from Mass/Volume using the volume of the soil core (308 cm^3) and the mass of the air-dried core.

Compaction of the cores was minimised when sampling by twisting the core into the soil.

(iv) Total nutrient contents

Sub-samples of air-dry soils were oven-dried to constant weight at 105 ^oC and percentage N, P, K and Ca contents determined as described in Section 2.2.2.

(v) Extractable nutrient contents

Air-dry soil (equivalent to 3 g dry weight) was shaken for 2 hours with 200 ml 0.1 N hydrochloric acid in a 250 ml polythene centrifuge bottle. After centrifuging (10 minutes at approximately 2000 rpm) the supernatant was filtered, the first few ml of filtrate being discarded. 5 ml of the remaining filtrate was made up to 50 ml with distilled water in a volumetric flask and retained for analysis.

The concentrations of P, K and Ca in the extract were determined as described in Section 2.2.2.1. Fe concentrations were determined directly by atomic absorption (Pye Unicam SP9). Al in the extract was determined using an automated colometric procedure employing the reaction with aluminon using glacial acetic acid/sodium hydroxide as a buffer, and in the presence of Thioglycollic acid to control interference by Fe (after Hsu, 1963).

The quantities of Fe and Al were considered on this occasion because Cuttle (1983) found that, in upland peats phosphate adsorption was closely related to the content of extractable iron and aluminium.

5.2.3 Results

5.2.3.1 Phosphorus bioassay

Results were analysed by analyses of variance using the statistical computing package, GENSTAT. Prior to statistical analysis, the data were checked for normality and homogeneity of variance. The whole ANOVA is presented in Appendix 5C but as

there is no replication of plots for either species or fertiliser regime, and hence, only one degree of freedom, no attempt is made to attribute statistical differences to these factors. Furthermore, there is no interaction of position with these factors and so the only comparisons made are between sampling positions within each sampling time.

Significant differences between individual means were established by Duncan's multiple range tests.

Figure 5.2.(i) and Appendix 5D present the mean ^{32}P uptake values in May, 1984. In the +PKCa Sitka spruce plot no differences were found between sampling positions, but in the -P plot, roots from the furrow took up less ^{32}P than those from the ridge position. In both lodgepole pine plots roots from the furrow took up less than those from the ridge and in the +PKCa plot, less than the flat as well.

There were no differences in uptake between the sampling positions in the +PKCa plots for either species in July, 1984 (Figure 5.2.(ii), Appendix 5D). In the -P plots, uptake increased in the order furrow, flat, ridge for both species.

In September, 1984 there were no position differences in either treatment plot for Sitka spruce (Figure 5.3(i), Appendix 5D). For lodgepole pine, the roots from the flat took up most ^{32}P in the +PKCa plot but in the -P plot, the roots from the furrow again took up the least ^{32}P.

Uptake was similar in roots from all sampling positions in all plots in February, 1985 (Figure 5.3(ii), Appendix 5D).

Climatic data from the nearest meteorological station to the site, corresponding both to the months of sampling, and the preceding and intervening months, are presented in Table 5.1, and discussed in relation to results (Section 5.4.1).

5.2.3.2 Soil Analysis

Significant differences ($P \geqslant 0.05$) between individual means within treatment plots were established by Duncan's multiple range tests, and all differences referred to are significant to at least this level.

(i) pH

No significant differences in pH between sampling positions were recorded in

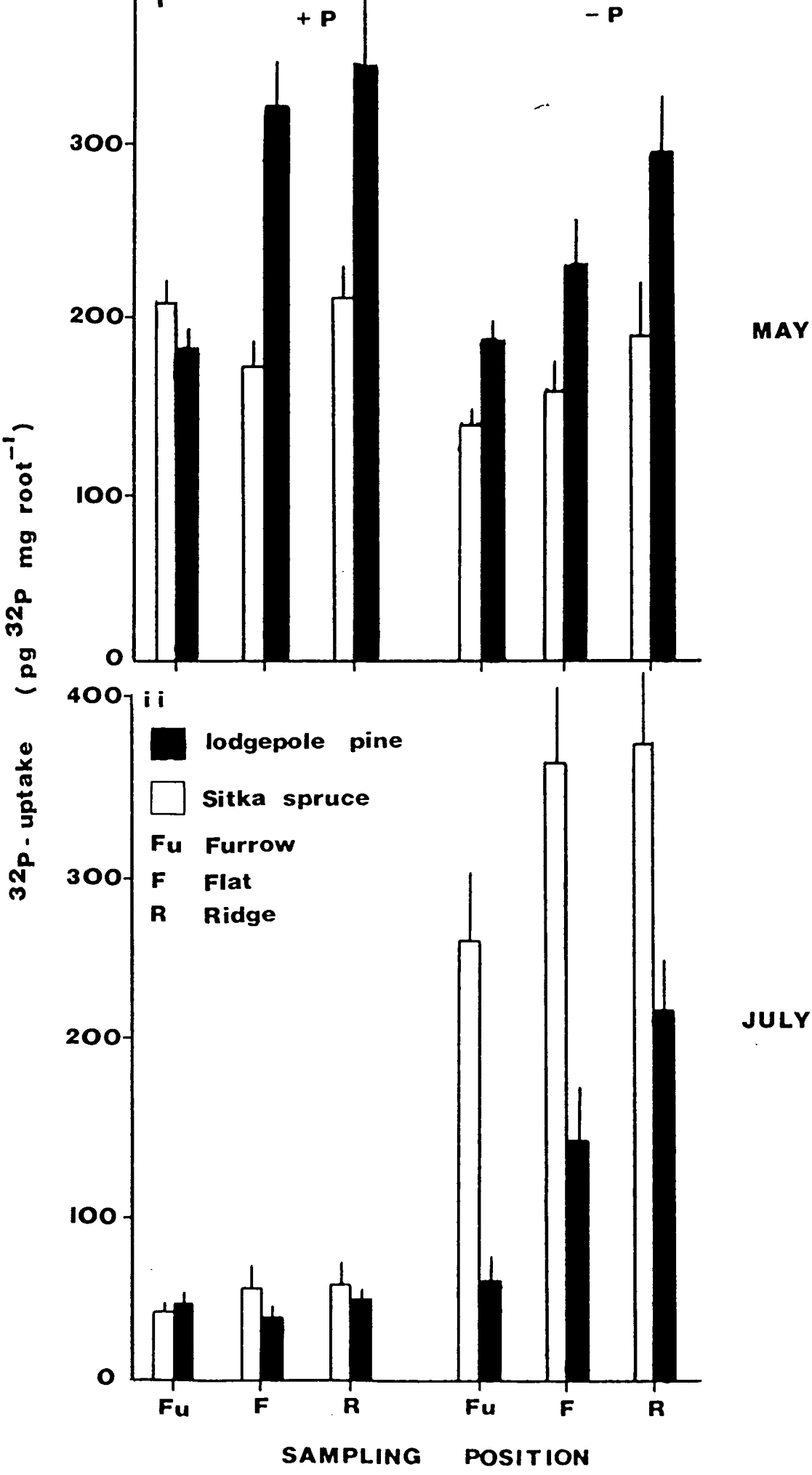


FIG. 5.2: Influence of sampling position on ^{32}P uptake in (i) May, 1984 (ii) July, 1984. ($\bar{x}$ + s.e., n = 10).

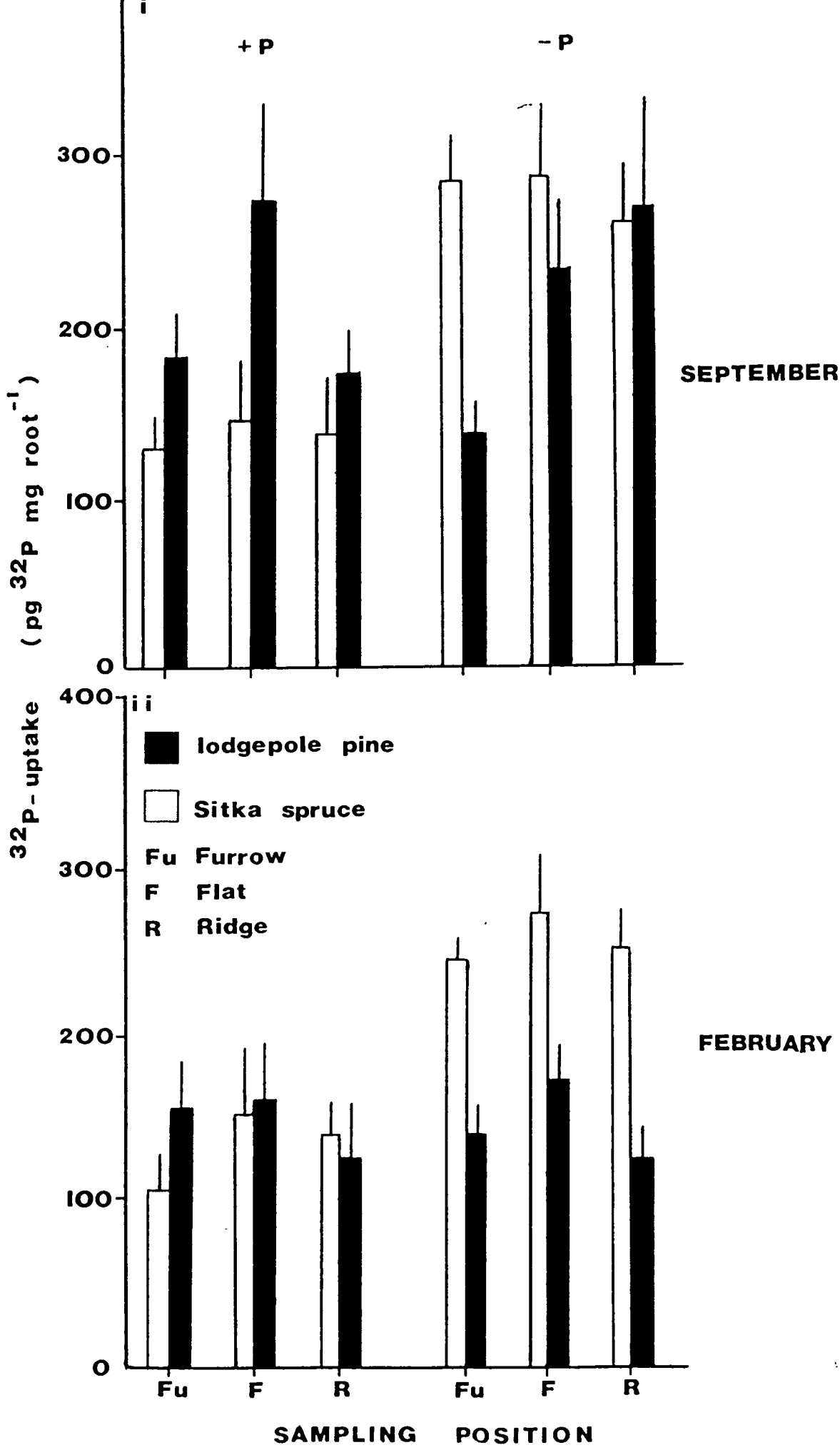


FIG. 5.3: Influence of sampling position on ^{32}P uptake in (i) September, 1984 (ii) February, 1985. ($\bar{x}$ ± s.e., n = 10).

TABLE 5.1: Climatic data from Bush House Meteorological Station
(5 miles East from site)

Month	Rainfall	Mean Air Temp.	Mean Soil Temp.
1984	**(mm)**	**(oC)**	**(^{o}C, 30 cm depth)**
March	117	3.3	4.1
April	20	6.5	6.7
May	47	8.7	10.7
June	59	12.3	13.3
July	26	15.0	15.7
August	29	14.6	15.8
September	87	11.3	13.1
October	98	8.9	9.9
November	194	6.3	7.0
December	63	4.3	4.8
1985			
January	33	0.7	1.7
February	18	2.1	2.0

any of the plots (Table 5.2(i)). The data indicates that the +PKCa plots were more acidic than the -P plots, for both species.

(ii) Moisture content

In all plots, except for lodgepole pine +PKCa, the furrows were considerably wetter than the ridges (Table 5.2(ii)). In the Sitka spruce plots, they were also wetter than the flats.

(iii) Bulk density

Table 5.3 shows that, in all plots, except for lodgepole pine -P, the furrows were less dense than the ridges.

(iv) Total nutrient content

Table 5.4 shows that the ridges are nutritionally poorer than the furrows in all plots. Exceptions arise for K concentrations in the +PKCa plots where no differences between the sampling positions were established and likewise for Ca concentrations in the -P plots.

(v) Extractable nutrients

The trends noted for total nutrient concentrations are not well reflected in the values of nutrients extracted from the soils of three sampling positions (Table 5.5 and 5.6). P status was similar in the lodgepole pine plots where, again less P was extracted from ridge soil than furrow soil. However, no differences in P were found in the Sitka spruce -P plot, and in the +PKCa, the flats contained the highest levels.

No differences in K levels were found, except in samples from the flat of the Sitka spruce +PKCa plot, which were higher than the other two positions.

Ca extracted from samples of the flat of the Sitka spruce -P plot were higher than the furrow or ridge, and levels from the furrow higher than the ridge or flat in the lodgepole pine +PKCa plot. However, no differences were established in the other two plots.

Quantities of Fe extracted from the flat area were highest in the lodgepole pine plots, but with Sitka spruce, the pattern is as before, with the furrows being richer in Fe than the ridges.

TABLE 5.2: (i) pH (ii) Moisture content (%) of soil samples from the furrow, flat and ridge sampling positions (mean values (n = 6) and (95 % C.I.)).

(i) pH	**+PKCa**	**-P**
Sitka spruce		
Furrow	3.1(0.3)	3.7(0.3)
Flat	3.3(0.3)	4.3(1.5)
Ridge	3.1(0.4)	3.6(0.6)
Lodgepole pine		
Furrow	3.1(0.6)	3.4(0.2)
Flat	2.9(0.3)	3.5(1.1)
Ridge	2.8(0.3)	3.9(1.8)

(ii) Moisture content (%)	**+PKCa**	**-P**
Sitka spruce		
Furrow	83.8(5.6)	84.5(3.8)
Flat	77.2(6.0)	75.8(5.7)
Ridge	74.5(8.3)	75.5(6.2)
Lodgepole pine		
Furrow	74.2(6.2)	72.4(9.8)
Flat	73.9(5.7)	73.4(6.5)
Ridge	74.7(5.9)	66.2(6.8)

TABLE 5.3: Bulk density (g cm^{-3}) of soil samples from the furrow, flat and ridge sampling positions. (Mean values (n = 6) and (95 % C.I.)).

	+PKCa	-P
Sitka spruce		
Furrow	0.11(0.04)	0.18(0.09)
Flat	0.14(0.04)	0.23(0.16)
Ridge	0.16(0.06)	0.25(0.11)
lodgepole pine		
Furrow	0.15(0.04)	0.26(0.16)
Flat	0.17(0.09)	0.25(0.10)
Ridge	0.21(0.06)	0.26(0.08)

TABLE 5.4: Total nutrient content (%) of soil samples from the furrow, flat and ridge sampling positions. (Mean values (n = 6) and (95 % C.I.)).

	P	N	K	Ca
+PKCa				
Sitka spruce				
Furrow	0.07(0.0)	1.21(0.25)	0.05(0.02)	0.21(0.12)
Flat	0.07(0.01)	1.08(0.47)	0.09(0.05)	0.32(0.28)
Ridge	0.04(0.02)	0.96(0.48)	0.05(0.05)	0.30(0.22)
Lodgepole pine				
Furrow	0.07(0.02)	1.19(0.28)	0.06(0.02)	0.31(0.23)
Flat	0.04(0.02)	0.99(0.16)	0.04(0.02)	0.29(0.15)
Ridge	0.03(0.01)	0.92(0.54)	0.04(0.05)	0.16(0.15)
	P	**N**	**K**	**Ca**
-P				
Sitka spruce				
Furrow	0.05(0.01)	1.58(0.39)	0.06(0.01)	0.52(0.25)
Flat	0.04(0.01)	1.31(0.27)	0.06(0.01)	1.00(1.03)
Ridge	0.03(0.02)	1.26(0.27)	0.05(0.04)	0.53(0.88)
Lodgepole pine				
Furrow	0.06(0.02)	1.40(0.36)	0.06(0.01)	0.46(0.17)
Flat	0.07(0.01)	1.42(0.27)	0.07(0.02)	0.69(1.05)
Ridge	0.04(0.01)	1.19(0.14)	0.04(0.02)	0.94(1.44)

TABLE 5.5: P, K, and Ca (mg g^{-1}) extracted from soil samples from the furrow, flat and ridge sampling positions. (Mean values (n and (95 % C.I.)).

	P	K	Ca
+PKCa			
Sitka spruce			
Furrow	0.07(0.08)	0.36(0.20)	2.36(3.13)
Flat	0.15(0.18)	0.78(0.61)	3.18(2.67)
Ridge	0.05(0.04)	0.50(0.56)	2.65(1.91)
Lodgepole pine			
Furrow	0.16(0.08)	0.48(0.19)	3.43(1.80)
Flat	0.08(0.13)	0.25(0.28)	2.77(1.39)
Ridge	0.06(0.10)	0.36(0.70)	1.61(1.32)

	P	K	Ca
-P			
Sitka spruce			
Furrow	0.03(0.01)	0.47(0.31)	4.71(3.52)
Flat	0.04(0.07)	0.37(0.15)	8.86(4.59)
Ridge	0.03(0.03)	0.36(0.37)	5.02(6.10)
Lodgepole pine			
Furrow	0.09(0.04)	0.46(0.34)	4.44(2.67)
Flat	0.11(0.07)	0.50(0.28)	6.53(3.42)
Ridge	0.04(0.02)	0.32(0.17)	9.06(4.36)

TABLE 5.6: Fe and Al (mg g^{-1}) extracted from soil samples from the furrow, flat and ridge sampling positions (Mean values (n = 6) and (95 % C.I.)).

	Fe	Al
+PKCa		
Sitka spruce		
Furrow	0.45(0.29)	0.12(0.17)
Flat	0.38(0.24)	0.02(0.03)
Ridge	0.18(0.06)	0.09(0.10)
Lodgepole pine		
Furrow	0.16(0.15)	0.14(0.09)
Flat	0.35(0.31)	0.06(0.08)
Ridge	0.14(0.05)	0.20(0.32)
	Fe	**Al**
-P		
Sitka spruce		
Furrow	0.43(0.30)	0.27(0.11)
Flat	0.34(0.16)	0.40(0.20)
Ridge	0.15(0.06)	0.37(0.21)
Lodgepole pine		
Furrow	0.38(0.22)	0.25(0.20)
Flat	0.47(0.19)	0.39(0.31)
Ridge	0.29(0.10)	0.32(0.06)

Levels of Al were highest in samples from the furrows in the two +PKCa plots, but in the -P plots, the furrows yielded the lowest levels.

5.3 THE INFLUENCE OF WITHIN-PLANT VARIABILITY ON THE BIOASSAY

5.3.1 Planting materials

Seedlings (1+0) of Queen Charlotte Island Sitka spruce and Skeena lodgepole pine were lifted from the Forestry Commission nurseries at Tulliallan on the 13th March 1985. They were stored in polythene bags in a cold room at 2 ^{o}C overnight. On 14th March, seedlings of uniform height and vigour were selected, having two roots of similar diameter arising close to the stem base. Unwanted roots were removed. Subsamples of such trees, 30 for each species, were taken for initial analysis.

The seedlings were then planted in 5" square pots, so that one root was positioned in either of two adjacent pots, filled with equal volumes of horticultural peat.

The pots were placed in saucers and the seedlings placed in an unheated glasshouse. After a month in the glasshouse, during which time the plants were supplied only with water, well-established plants were chosen for the experiment.

On 20th April, four treatments were designated plus two controls, and twenty-two plants for each species received phosphorus as sodium dihydrogen phosphate at rates equivalent to 5, 10, 20 and 50 kg ha^{-1} elemental phosphorus to one of the pots containing a root. The control plants received either no phosphorus to either pot or phosphorus at a rate equivalent to 50 kg ha^{-1} to both pots (Figure 5.4).

In addition, all the pots including the controls, received the equivalent of 100 kg ha^{-1} elemental K as potassium chloride and 40 kg ha^{-1} elemental N as ammonium nitrate. The nutrients were supplied in solution.

The plants were arranged in twenty-two randomised blocks in the greenhouse, each block containing one plant for each treatment and one of each control, for each species.

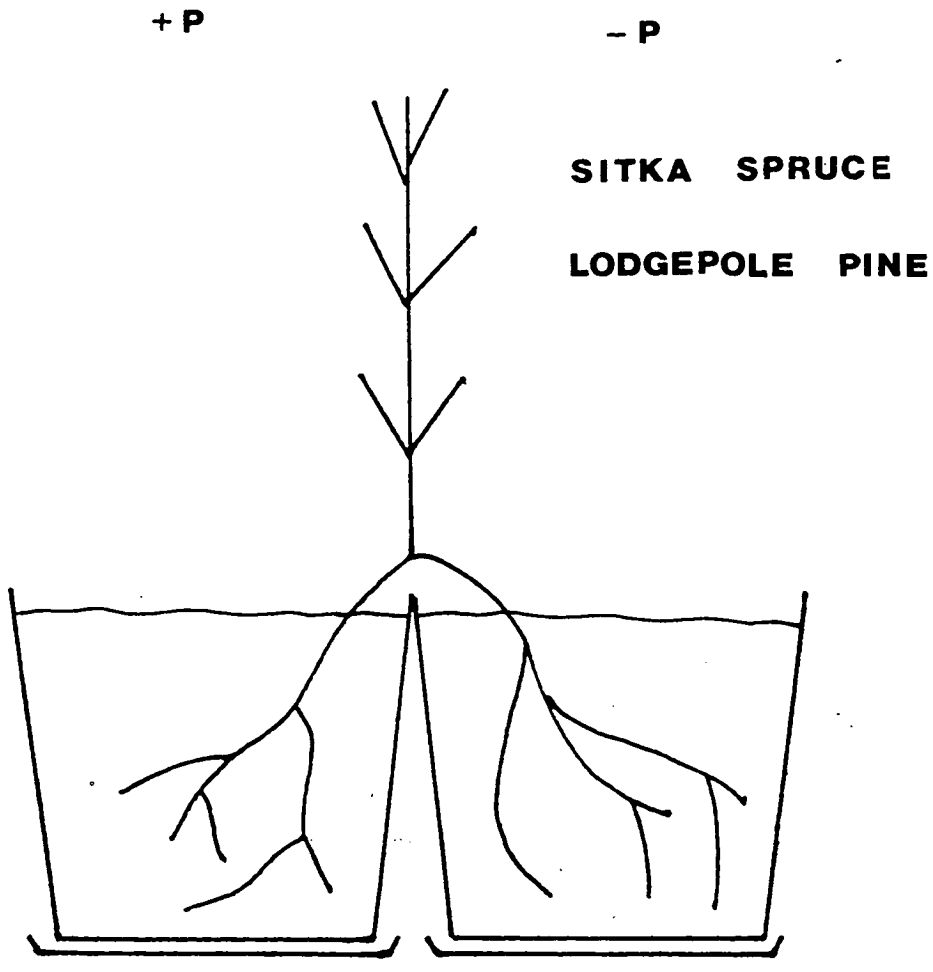


FIG 5.4: Rates of phosphorus fertiliser application.

The plants were left, adequately watered, but with no further fertilisation over one growing season, until harvesting in November 1985.

5.3.2 Sampling, measurements and analysis

(i) Height

The heights of the seedlings, from the root collar at the peat surface to the highest point on the leading shoot were recorded at the start of the experiment before fertilisation and at the end, on the 30th October, 1985.

The height difference is expressed as the height increment over the experimental period.

(ii) Root collar diameter

Root collar diameter was measured immediately above the peat level using a caliper divided to 0.01 mm at the start of the experiment before fertilisation and at the end, on the 30th October, 1985. The increment in root collar diameter is expressed as before.

(iii) Harvest and plant handling

The seedlings were harvested intact on 12th November, 1985 and the roots carefully teased from the peat in each pot and individually labelled. The root systems were then washed under running tap water and segments of approximately 10 cm length subsampled from each root of the plant, labelled and stored under moist tissue papers in a cool room.

The remainder of the plant was dried in an air-circulation oven at 105 ^{o}C to constant weight, and then stored in paper bags in a cupboard.

The peat from both pots was air-dried at 35 ^{o}C and stored in paper bags in a cupboard.

(iv) Plant dry weight

After oven-drying to constant weight at 105 ^{o}C, each plant was separated into separate root systems, stem and needles and each component weighed. The weight of 50 needles was also recorded, and the 1000 needle weight calculated. The representative sub-sample of plants used, initially collected and dried in April, was

treated in the same way.

Mean initial values for root, stem, needle and 1000 needle weight were calculated.

Final results are expressed as the weight increment for the stem and needle weights, over the experimental period. However, since some root was removed at planting, and also at harvesting when small sub-samples were removed for bioassay, it was not possible to express root weights similarly. Therefore, root weights are given as the final total weight of both root systems and as the difference in weight between them, the value for the non-fertilised root being subtracted from that of the fertilised root.

(v) Plant nutrient content

The stems, needles and both root systems were bulked by treatment and species. The bulked samples were ground in a hammer mill and total N, P and K determined as described in Section 2.2.2 for six sub-samples of each treatment. The initial subsamples were treated in the same way, and results are expressed as nutrient uptake.

(vi) Soil nutrient content

After air-drying, the peat from each pot of the respective treatment for each species was bulked. The bulked samples were sieved and ground as described in Section 5.2.2.3 and total N, P, K and Ca contents and extractable P, K, Ca, Fe and Al determined for six subsamples, as in Section 5.2.2.3.

(vi) Phosphorus bioassay

The lengths of fresh root subsampled at harvesting were bioassayed the next day as in Section 2.2.2.

5.3.3 Results

Results were analysed by analyses of variance using the statistical computing package, SPSS. Prior to statistical analysis, the data were checked for normality and homogeneity of variance.

Nutrient analyses for both plant tissue and peat were not, however, analysed in this way. By bulking the blocks, the measure of between plant variability is removed.

However, the six sub-samples taken showed little measurement error or variability due to lack of mixing, and so, trends can be observed for the various treatments.

In the following tables, the treatment key is as follows;

P0/0 - 0 kg P ha^{-1} applied to either pot

P5 - 5 kg P ha^{-1} applied to one pot

P10 - 10 kg P ha^{-1} applied to one pot

P20 - 20 kg P ha^{-1} applied to one pot

P50 - 50 kg P ha^{-1} applied to one pot

P50/50 - 50 kg P ha^{-1} applied to both pots

and, when the data refer to either a root system, or the peat, from one pot of the treatment;

P0/0 - As before

P0/5 - 0 kg P ha^{-1} applied to particular pot, but 5 kg P ha^{-1} applied to its partner pot.

P5/0 - 5 kg P ha^{-1} applied to particular pot, but 0 kg P ha^{-1} applied to its partner pot.

etc.

(i) Height increment

The mean values for each treatment are presented in Table 5.7.

Analyses showed that, for lodgepole pine, there were no significant differences in height increment between any of the treatments. With Sitka spruce, however, the P0/0 trees grew less than any of the other treatments and P5 grew less than the two highest P treatments, P50 and P50/50.

(ii) Root collar diameter increment

The mean values for this parameter are also presented in Table 5.7.

The treatment differences in Sitka spruce were the same as for height increment. Lodgepole pine trees showed a greater sensitivity to the treatments in that, both P0/0 and P5 showed less increment than any of the other treatments. P10 and P20 were similar and both less than P50 and P50/50, which were also similar.

TABLE 5.7: Height increment (HI) (mm) and root collar diameter increment (RCDI)(mm) over the experimental period (Mean values (n = 22) and (95 % C.I.)).

Sitka spruce

	HI	RCDI
P0/0	138.9 (48.9)	3.1 (0.4)
P5	171.3 (46.2)	4.3 (0.5)
P10	200.0 (43.7)	4.6 (0.2)
P20	199.2 (30.5)	4.7 (0.3)
P50	206.1 (34.0)	4.8 (0.4)
P50/50	201.8 (37.2)	5.3 (0.3)

lodgepole pine

P0/0	59.5 (30.8)	2.9 (0.6)
P5	53.6 (24.6)	3.3 (0.4)
P10	71.1 (24.5)	3.8 (0.2)
P20	66.5 (26.7)	3.9 (0.3)
P50	73.4 (27.7)	4.2 (0.2)
P50/50	66.5 (24.2)	4.2 (0.4)

(iii) Plant dry weight

The treatment effects on the increase in above-ground biomass were not marked, as shown in Table 5.8. There were no significant differences in the increase in any of stem weight, needle weight or 1000 needle weight for the lodgepole pine seedlings. For Sitka spruce, the increases in stem weight and needle weight for the P0/0 plants were less than for the other treatments, but otherwise, no differences were observed.

Total root production was similar in all treatments, except for P0/0 which was significantly less than all other treatments in Sitka spruce, and less than the highest P applications, P50 and P50/50 in lodgepole pine (Table 5.9).

The difference in production between fertilised and non-fertilised root systems is best considered by comparison with the controls, which produced more or less equal amounts of root on both sides (Table 5.9). This was also true for all the Sitka spruce plants, no treatment differences were detected, or weight differences between the two root systems. With lodegepole pine, plants of the P10 treatment showed a weight difference inconsistent with the controls, indicating more root production in the fertilised pot.

(iv) Plant nutrient content

Increasing P applications appear to have had little effect on N uptake into above ground biomass for both species, (Tables 5.10 and 5.11) although foliar uptake is low in the P0/0 Sitka spruce plants. Uptake of P and K into the stem and needles both show an increase with increasing P applications.

The root nutrient contents, however, show disparity between the species. The difference in nutrient content of fertilised and non-fertilised roots in Sitka spruce is small, but in lodgepole pine, non-fertilised roots contain considerably less N, P and K than the fertilised roots (Table 5.12).

v) **Soil nutrient content**

The total nutrient contents of the peat differ little for the fertilised and non-fertilised pots in either species (Table 5.13).

Quantities of P extracted with 0.01M HCl are higher, however, in the fertilised

TABLE 5.8: Stem weight increment (SWI)(g), Needle weight increment (NWI)(g) and 1000 needle weight increment (KNWI(g)) over the experimental period. (Mean values (n = 22) and (95 % CI)).

Sitka spruce	**SWI(g)**	**NWI(g)**	**KNWI(g)**
P0/0	2.4 (1.0)	3.4 (1.4)	0.25 (0.3)
P5	3.1 (0.9)	4.5 (1.3)	0.24 (0.2)
P10	3.6 (0.9)	5.0 (1.2)	0.25 (0.1)
P20	3.4 (0.8)	5.0 (1.1)	0.33 (0.2)
P50	3.3 (0.7)	4.3 (0.9)	0.37 (0.3)
P50/50	3.0 (0.7)	4.5 (1.2)	0.36 (0.3)
lodgepole pine			
P0/0	1.8 (0.6)	4.0 (1.6)	14.5 (6.8)
P5	1.8 (0.5)	4.4 (1.1)	15.7 (4.0)
P10	2.1 (0.7)	4.3 (1.5)	16.2 (4.5)
P20	2.0 (0.5)	4.7 (1.4)	17.3 (4.6)
P50	2.1 (0.5)	4.6 (0.9)	15.6 (4.1)
P50/50	2.2 (0.8)	4.7 (1.1)	15.6 (3.9)

TABLE 5.9: Total root weight (RTOT)(g) and Weight of fertilised root-weight of non-fertilised root (RDIFF)(g) at end of experimental period. (Mean values (n = 22) and (95 % C.I.))

Sitka spruce	**RTOT(g)**	**RDIFF(g)**
P0/0	3.4 (0.9)	-0.15 (0.3)
P5	4.3 (0.9)	0.03 (0.8)
P10	4.7 (0.9)	-0.10 (0.7)
P20	4.6 (1.1)	0.06 (0.6)
P50	4.3 (1.1)	0.01 (1.0)
P50/50	4.4 (0.8)	0.09 (0.3)
lodgepole pine		
P0/0	4.8 (1.6)	-0.02 (0.8)
P5	5.0 (1.4)	0.50 (0.8)
P10	5.3 (1.7)	0.85 (1.0)
P20	5.4 (1.5)	0.28 (0.9)
P50	6.1 (1.3)	0.69 (1.2)
P50/50	6.0 (1.4)	0.01 (0.6)

TABLE 5.10: Nutrient uptake by stems over the experimental period (mg) (Mean of 6 subsamples).

Sitka spruce	**N**	**P**	**K**
P0/0	8.99	0.36	9.04
P5	9.34	0.77	11.99
P10	10.37	1.62	14.78
P20	9.95	2.51	15.99
P50	8.79	2.76	15.27
P50/50	8.01	3.73	16.20
lodgepole pine			
P0/0	8.42	0.76	8.43
P5	6.81	0.65	8.55
P10	8.82	1.36	10.21
P20	8.71	2.18	10.94
P50	10.03	2.67	11.42
P50/50	8.95	4.43	11.65

TABLE 5.11: Nutrient uptake by needles over the experimental period (mg). (Mean of 6 subsamples).

Sitka spruce	**N**	**P**	**K**
P0/0	37.88	3.76	41.32
P5	50.30	5.02	52.33
P10	51.40	7.12	65.38
P20	48.56	9.37	67.50
P50	44.23	9.75	60.60
P50/50	42.59	12.20	62.38
lodgepole pine			
P0/0	42.38	3.38	37.96
P5	43.00	4.26	45.79
P10	43.65	4.84	38.82
P20	42.72	6.67	48.85
P50	46.23	8.03	53.37
P50/50	45.70	9.66	54.36

TABLE 5.12: Nutrient content of roots at end of experimental period (mg) (Mean of 6 subsamples).

Sitka spruce	**N**	**P**	**K**
P0/0	7.94	0.93	7.75
P0/5	10.90	1.48	10.19
P0/10	11.30	2.13	11.81
P0/20	10.68	2.45	11.67
P0/50	10.71	2.64	11.27
P5/0	10.87	1.85	10.14
P10/0	10.51	1.96	10.36
P20/0	11.24	2.69	12.71
P50/0	10.19	2.89	10.95
P50/50	10.12	3.07	10.68
lodgepole pine			
P0/0	13.86	1.59	22.02
P0/5	11.21	1.31	19.72
P0/10	10.49	1.68	21.89
P0/20	11.63	3.24	27.02
P0/50	12.02	4.05	24.99
P5/0	14.73	1.99	26.62
P10/0	15.79	3.98	28.56
P20/0	14.69	5.59	27.15
P50/0	18.73	7.79	34.09
P50/50	15.27	8.00	30.71

TABLE 5.13: Total nutrient content (%) of peat.
(Mean of 6 subsamples)

Sitka spruce	**N**	**P**	**K**	**Ca**
P0/0	0.78	0.02	0.09	0.17
P0/5	0.72	0.02	0.09	0.20
P0/10	0.72	0.02	0.10	0.22
P0/20	0.75	0.02	0.09	0.19
P0/50	0.74	0.03	0.10	0.19
P5/0	0.70	0.02	0.10	0.22
P10/0	0.72	0.02	0.07	0.19
P20/0	0.80	0.03	0.09	0.21
P50/0	0.76	0.03	0.07	0.22
P50/50	0.75	0.04	0.09	0.21
lodgepole pine				
P0/0	0.80	0.02	0.08	0.19
P0/5	0.79	0.02	0.08	0.17
P0/10	0.75	0.02	0.08	0.18
P0/20	0.82	0.02	0.07	0.20
P0/50	0.79	0.02	0.07	0.17
P5/0	0.70	0.02	0.10	0.22
P10/0	0.79	0.02	0.07	0.19
P20/0	0.77	0.03	0.06	0.18
P50/0	0.78	0.03	0.07	0.20
P50/50	0.75	0.04	0.08	0.23

pots, the P50/50 pots yielding the largest amounts. For K, however, the opposite is true and little difference is observed for levels of Al, Fe and Ca (Table 5.14).

(vi) Phosphorus bioassay

The mean values of ^{32}P uptake for the two species are presented in Figures 5.5 and 5.6 and Appendix 5E.

The data showed heterogeneity of variances and required a $\log_{10}$ transformation before statistical differences could be established. The figures presented are, however, original data.

For both species, there were no differences in ^{32}P uptake between the fertilised and non-fertilised roots. Uptake was highest in the P0/0 treatment for both species, but similar to the P5 treatment. In Sitka spruce, uptake by roots from the four highest P treatments was similar and in lodgepole pine, roots from P50/50 and P50/0 took up similar amounts of ^{32}P and P0/50 roots and those from the intermediate treatments of P20 and P10 showed similar uptake.

5.4 DISCUSSION

5.4.1 Spatial Variability of the Forest Floor

The sampling programme at Eddleston indicates that roots from different positions on the forest floor vary in their degree of phosphorus requirement, shown by differential uptake of ^{32}P.

The response is not consistent however, between season, or phosphorus fertilisation regime.

The seasonal variations are similar to those observed at Wauchope (Chapter 4), an increase in ^{32}P-uptake concurring with the increasing soil temperatures and rainfall observed in the early part of the year (Table 5.1), and hence presumably nutrient availability. This effect was most marked in July in the -P Sitka spruce plot. The high demand of the tree following the spring, a period of physiological activity, was probably not met, this being the only plot without canopy closure. Consequently, there will be little internal nutrient cycling in this stand (Miller, 1984). The nutrient

TABLE 5.14: Nutrients extracted (mg g^{-1}) from peat.
(Mean of 6 subsamples).

Sitka spruce	**P**	**K**	**Al**	**Fe**	**Ca**
P0/0	0.02	0.95	0.31	0.17	1.68
P0/5	0.01	1.06	0.29	0.18	1.82
P0/10	0.02	1.04	0.31	0.22	1.91
P0/20	0.02	0.86	0.33	0.23	1.75
P0/50	0.04	1.03	0.31	0.18	1.80
P5/0	0.01	0.93	0.30	0.31	1.84
P10/0	0.03	0.71	0.31	0.26	1.86
P20/0	0.03	0.77	0.35	0.25	1.80
P50/0	0.08	0.68	0.30	0.21	1.92
P50/50	0.14	0.97	0.32	0.20	1.90
lodgepole pine					
P0/0	0.02	0.74	0.31	0.25	1.71
P0/5	0.01	0.73	0.33	0.24	1.71
P0/10	0.01	0.79	0.32	0.29	1.74
P0/20	0.01	0.72	0.35	0.26	1.72
P0/50	0.01	0.76	0.29	0.24	1.68
P5/0	0.01	0.66	0.29	0.26	1.72
P10/0	0.01	0.57	0.34	0.34	1.70
P20/0	0.04	0.67	0.33	0.25	1.67
P50/0	0.11	0.65	0.32	0.25	1.75
P50/50	0.19	0.85	0.34	0.24	1.75

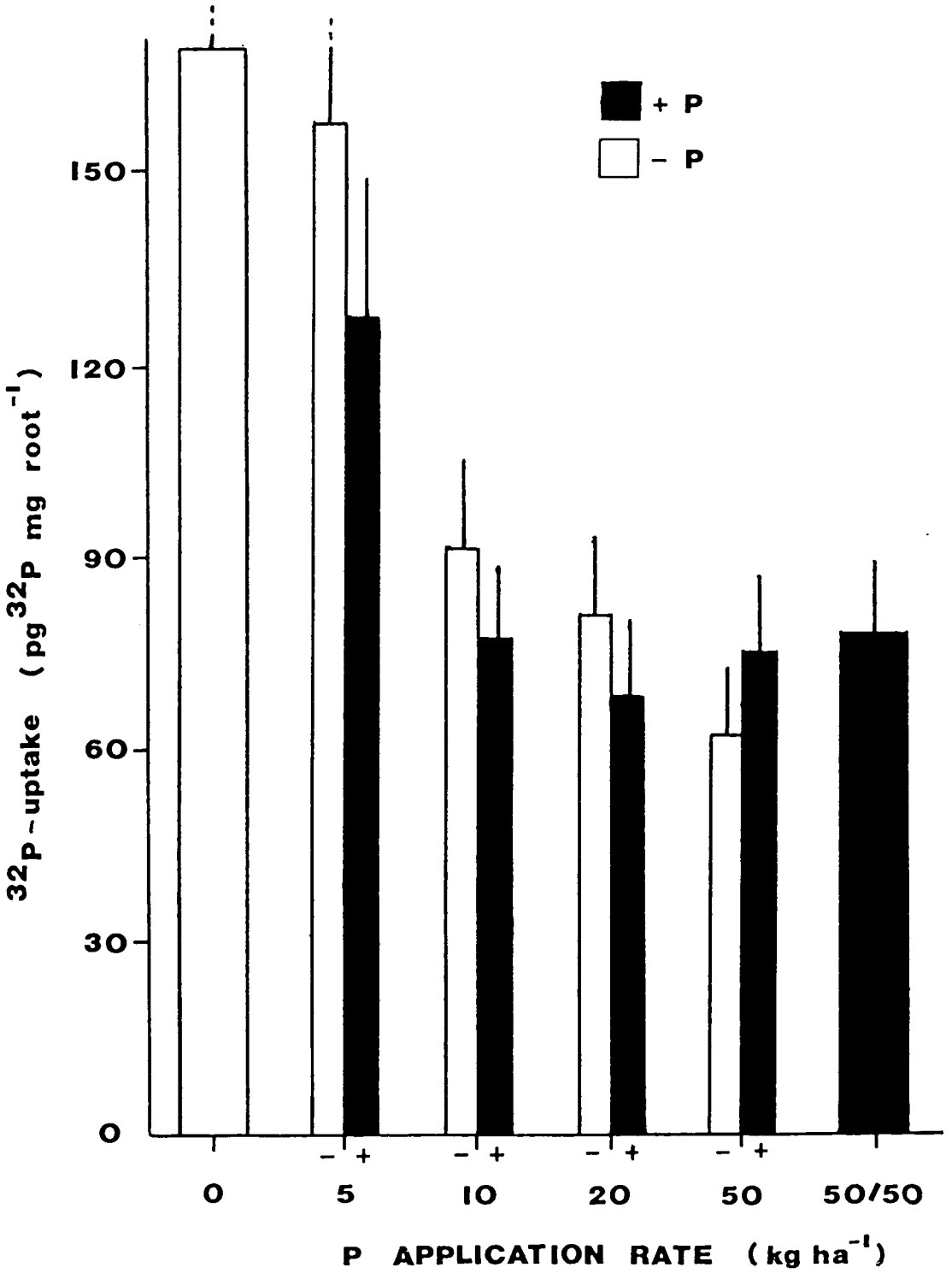


FIG. 5.5: Uptake of ^{32}P, by Sitka spruce divided root systems ($\bar{x}$ + s.e., n = 22).

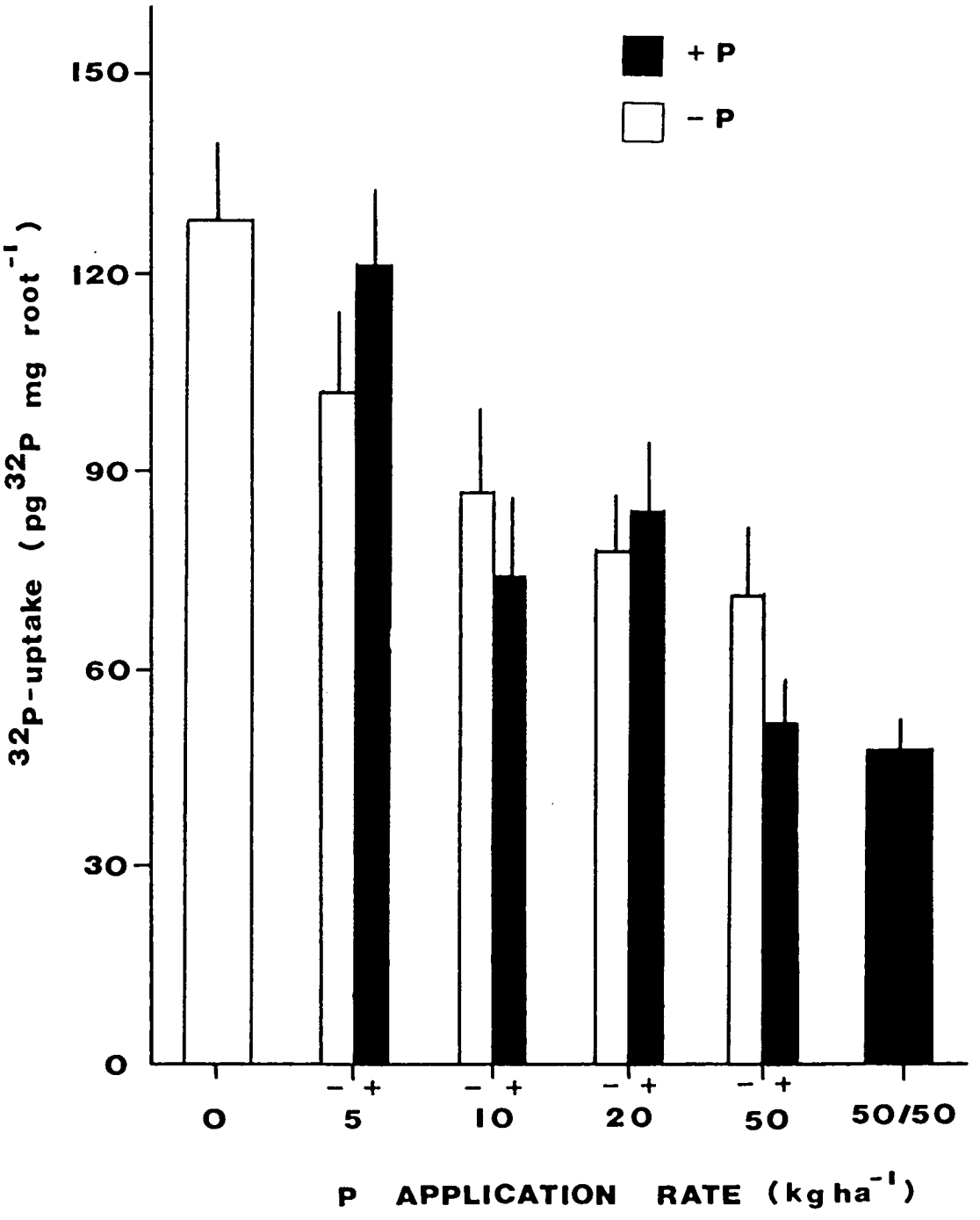


FIG. 5.6: Uptake of ^{32}P by lodgepole pine divided root systems. ($\bar{x}$ + s.e., n = 22).

supply of the forest floor will not be sufficient for the trees needs, indicated by high ^{32}P uptake.

Positional differences, where they are found, indicate that the furrow is the region of highest phosphorus availability, and the ridge the least. The July sampling period appeared the most sensitive to the positional differences, in the -P plots for both species, and also showed the greatest differences between fertilised and non-fertilised plots. For these reasons, the soil cores for analysis were collected in July of the following year.

The soil analyses, to some extent, verify the bioassay results. Total nutrient contents of the soil cores show the ridge to be nutritionally poorer than the flat or furrow for phosphorus and nitrogen. Potassium and calcium contents show less stratification, but this is not surprising as these nutrients are readily leached from the soil profile and do not usually accumulate (Malcolm and Cuttle, 1983).

The levels of phosphorus extracted from the respective positions were, in general, low and differences were inconsistent. Cuttle (1983) found phosphorus to be less available in peaty soils containing high levels of iron and aluminium due to the formation of hydroxy phosphates of these elements. The -P plots showed higher levels than the +P plots of both these elements for lodgepole pine and higher Al content, although not Fe, in the Sitka spruce plots. This may have caused a tie-up of available P.

The general indication is, however, that the ridges are nutritionally impoverished with respect to the other two positions, in particular to the furrows.

Similarly, the ridges and furrows showed the extremes of physical soil factors. The ridges were generally drier than the furrows and consequently more dense. This is probably due to run-off from the ridges which is increased by at least four factors; 1) the shape of the ridges, 2) relatively smooth and waxy mats of needles (also true for the flats), 3) the layered structures within the ridges that would delay penetration, and 4) the presence of dry peat which is only rewetted with difficulty (Deans, 1979). However, Deans found the maintenance of fine root populations was optimal in the

ridge in his study, because frequent moisture charges in this region were permitting a relatively abundant supply of nutrients. This was obviously not the case at the time sampled at Eddleston. When rainfall is low, and stemflow is not sufficient to recharge moisture levels on the ridges, mineralisation rates and hence nutrient availability is low. Surface rooting will not be favoured, and may even result in root mortality. At this time, the furrows are likely to be the areas of highest availability due to the lower soil moisture tension. However, at times of high precipitation, particularly when sustained over the winter months, when the ground water table is high, anaerobic conditions in furrows may result in root mortality (Coutts and Philipson, 1978; Lieffers and Rothwell, 1987). This was not observed in February 1985, following a winter of relatively low rainfall (Table 5.1).

The implications for the bioassay would seem to be that the flat is the most suitable sampling position. Being in an intermediate position, it is less susceptible to the run-off observed from the ridge and is probably influenced to a lesser extent by fluctuating incident precipitation to maintain fine root populations. Equally, the flat is not prone to waterlogging in winter as is the furrow and will therefore show less root mortality.

5.4.2 Within-plant variability

The results of the split root experiment indicate that the P variability within the root systems was not of a magnitude to be detected by the bioassay. This was verified to some extent by the other variables considered in the experiment.

That the different rates of phosphorus fertilisation did have some effect is demonstrated by the reduced increment in the height and root collar diameter of non-fertilised seedlings and those grown under the lowest fertiliser regimes. However, reduced growth was only observed in plants receiving the lowest rates of phosphorus application, or non-fertilised (P) plants and this effect is observed in both above and below ground production. That is, the different rates of phosphorus application did not significantly affect growth. This is partly because at the end of the experimental period, plants receiving higher P rates were not particularly P stressed

(Table 5.15). In addition, at the start of the experiment, the plant major nutrient concentrations were optimal for all nutrients. By the end of the experimental period, the plants were still optimal for K, but were showing signs of nitrogen stress, reflected in needle N content. This was particularly so for plants receiving higher rates of P. So, in the non P fertilised and lower P applications, phosphorus may have been the limiting factor. Plate 5.1 illustrates foliar symptoms of P stress in lodgepole pine seedlings which were not evident at higher rates of P application. These symptoms were also evident in non or P5 fertilised Sitka spruce. In the plants receiving higher rates of P, N may have become limiting. Plate 5.2 illustrates foliar symptoms of N stress which were not evident in the lower P treatments.

No real differences were found in production between the fertilised and non-fertilised root systems. This is not in keeping with other workers' results, for example, Coutts and Philipson (1976) and McClure (1972) who found that the favoured sides of divided root systems grew better, while Kimmins and Hawke (1978) attributed variations in the vertical plane in root densities to be positively correlated with soil fertility, and so too did Drew *et al.* (1973) in relation to nitrate concentrations in the soil.

However, despite the lack of differences in root weight, it appeared that non-fertilised roots were longer per unit soil volume, although this was not quantified. Plate 5.1 shows that, in lodgepole pine, the non-fertilised root system was longer, even in the lowest P treatment, and non-fertilised roots were considerably longer than most of the fertilised systems in most treatments. The effect was also observed in Sitka spruce (Plate 5.2) the example shown illustrates the effect at the highest P treatment and shows the short root systems when both systems were fertilised. This is likely to be a consequence of lower nutrient levels in the rooted soil volume and hence, more resources diverted to 'seeking' roots. This is partly borne out by the soil analyses. Although no differences were observed in total soil nutrient contents, the K levels extracted were lower in the non-fertilised pots, even though equal amounts had been applied to both sides - the non-fertilised roots, having possibly exploited a greater

TABLE 5.15 (i) Nutrient content of Needles after experimental period (%). (Mean of 6 subsamples),
(ii) Initial content of needles (%) $\bar{x}$, n = 32 and (s.e.))

(i) Sitka spruce	**N**	**P**	**K**
P0/0	1.18	0.12	1.22
P5	1.17	0.12	1.17
P10	1.06	0.15	1.29
P20	1.02	0.19	1.35
P50	1.07	0.23	1.39
P50/50	0.99	0.27	1.37
lodgepole pine			
P0/0	1.14	0.09	0.94
P5	1.07	0.11	1.04
P10	1.06	0.12	0.87
P20	1.01	0.15	1.05
P50	1.07	0.18	1.13
P50/50	1.05	0.21	1.14

	N	**P**	**K**
(ii) Sitka spruce	2.31 (0.04)	0.29 (0.01)	1.04 (0.04)
lodgepole pine	2.15 (0.03)	0.24 (0.00)	0.80 (0.02)

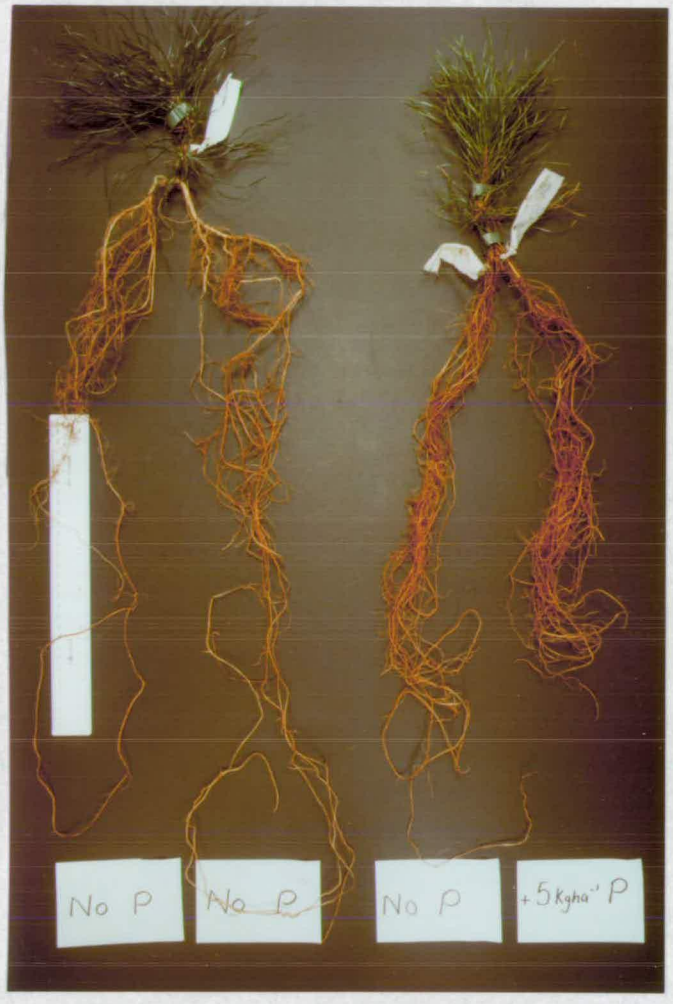


PLATE 5.1: Effect of Differential P Application on Root Morphology in Lodgepole Pine.

PLATE 5.2: Effect of Differential P Application on Root Morphology in Sitka Spruce.

soil volume, showed greater efficiency in uptake. This effect was not paralleled for Al, Fe and Ca, but these elements are less mobile.

There also seems to have been a species difference in uptake. The lodgepole pine seedlings had higher root P contents than Sitka spruce at the end of the experimental period, while Sitka spruce showed higher foliar contents. Furthermore, fertilised lodgepole pine roots contained higher P levels than the non-fertilised roots, which was not the case with Sitka spruce. This would imply that, either 1) Sitka spruce translocated P more efficiently to the shoot, or 2) P was translocated to deprived roots in Sitka spruce. There was obviously a degree of translocation in both species, however, in that non P fertilised root systems attached to fertilised plants all showed higher P concentrations than the control plants, increasingly so with higher rates of P application (Table 5.12).

In either case, the bioassay did not indicate for either species, that the non-fertilised roots were P deficient in comparison to the favoured roots. It did show, however, that the non-fertilised seedlings were more deficient than those which had been fertilised. That is, spatial variability within the plant was not detected by the bioassay, either because the variability was reduced by internal translocation, or because, considering the high P plant levels at the beginning of the experiment, the experiment did not run for long enough to induce significant effects. Either way, the implications for sampling would appear to be that variability within the horizontal plane of the 'flat' position is of lesser importance than the spatial variability of the stratified forest floor. Consequently, identification of the parent tree of fine roots samples is of less importance than standarisation of the sampling position.

CHAPTER 6

DISCUSSION

The extent to which the bioassay may integrate complex biological factors to give a unified picture of the phosphorus status of tree crops and its potential as a predictor of fertiliser requirement is assessed in this section.

The initial studies were encouraging; the greenhouse experiment presented in Chapter 3, showed that the ^{32}P uptake by roots under bioassay conditions is a negative function of the phosphorus supply in the soil, bearing out the earlier findings by Harrison and Helliwell (1979). Furthermore, the bioassay was an equally good, if not better, indicator of P status than the P content of plant tissue, estimated by conventional chemical means. Throughout the project, the same negative relationship was established between applied phosphorus and ^{32}P uptake.

However, in the other experiments, although these relationships were consistent, the variability of results occasionally was considerable. Suggestions were made as to the source of variation, but generally the conclusion was that several factors were interacting.

The major source of variation seems to arise from the fact that the uptake of ^{32}P in the bioassay is metabolically mediated and is therefore dependent on the metabolic state of the plant at the time of sampling. Hence the considerable seasonal variation observed, uptake values varying by a factor of about four, over the season sampled. As discussed in Chapter 4, attempts could be made to minimise this by sampling at times of year which avoid climatic extremes, but there is generally considerable year to year variations in climate. This was partly demonstrated by the three fold increase in ^{32}P uptake in April 1985 compared with April 1984. The magnitude of this type of variation would have to be examined over several seasons before it could be accounted for in a routine sampling programme. However, it was assumed that the bioassay integrated the effects of the various processes which are simultaneously influenced by climate, such as mineralisation rates affected by enzymatic activity and microbial

populations. Thus, if the variation in bioassay results could be explained as a consequence of climatic differences over several seasons, as they were over one season, then it should be possible to recognise optimal sampling times.

An area not considered during this project is the mechanism of the increased uptake in stressed plants. This 'hunger' effect is a phenomenon often observed in plant physiological studies but never fully understood because of the many factors which may contribute to it. Mycorrhizal infection is well known to be greater on phosphorus deficient roots and to enhance P uptake. Mycorrhizas were regarded as a 'black box' for this project - judged to be an integral part of phosphorus deficient plants. In the field situation, fine roots are predominantly mycorrhizal and represented by mixed populations of mycorrhizal fungi - the effects of which would have been difficult to isolate. For instance, Mejstrik and Krause (1973) showed that *Suilleus luteus* had a stimulatory effect on P-uptake of *Pinus radiata* seedlings, but that *Cenococcum graniforme* had a slightly inhibitory effect over non-mycorrhizal fungi. In addition, Harrison and Helliwell (1979) found that a consideration of percentage mycorrhizal association on birch seedlings did not account for any additional variation beyond that accounted for by soil phosphorus properties.

Similarly, the role of characteristics of root distributions and morphology have not been considered. Barr and Ulrich (1963) found that in high and low phosphate *Phaseolus limensis* var. Fordhook conc. (Lima bean) plants, there was a very large variation in orthophosphate content, reflecting the external phosphate supply. The implications for this were that stressed plants had an enhanced ability to take up orthophosphate. It has been observed that plants poorly supplied with P have higher root carbohydrate levels than roots adequately supplied with P. (Mengel & Kirby, 1982). High carbohydrate levels are thought to be a prerequisite for good mycorrhizal infection. The heterogeneity of fine roots in the field and associated nutrient availability is complicated by the range of processes which occur at the root surface, and which may greatly increase the potential rate of uptake of nutrients. These include root hairs, pH change and the excretion of complexing ligands. The variability

induced by the reaction of the root system to such heterogeneity would be difficult, if not impossible, to isolate and quantify in a field situation. However, it would seem that the bioassay is sufficiently robust to account for this unknown variability. It was demonstrated in Chapter 2 that ten roots sampled per plot accounted for similar variability as did fifty roots. Chapter 5 demonstrated that spatial variability in ploughed sites would be allowed for, and that the bioassay, under controlled conditions, integrated the effects of differing P supply in the rooting environment. This means that, at one time, within a site, assessment of P status can be made by comparison of treatment plots. This project has not indicated, however, the potential of assessing P status in one year on one site compared with another year on another site. This project has involved the comparison, at one time, of fertilised and non-fertilised trees, and with the results discussed in relation to each other. Given the magnitude of the spatial and temporal variability observed in the sites studied, it has not been possible to obtain critical values of ^{32}P uptake which would indicate a response could be obtained to the application of P fertiliser. Investigation of this would necessitate compilation of data banks, involving testing the bioassay in stands growing on sites of different soil type, age, altitude, of various species and other macro-site variables. This would require the establishment of long-term field studies.

It was beyond the scope of this project to consider growth response to applied fertiliser. Sampling by Harrison *et al.* (1987, in press) from an age series of Sitka spruce, varying from 0.5 to 16 m in height and in age from 1 - 33 years considered this. In 1982, roots were taken from each of the 24 stands and assessed by the bioassay procedure. The roots showed a wide range of variation in ^{32}P uptake, the highest values being for trees between 7 and 11 m. This was not reflected by the concentration of phosphorus in the tree needles. To test the hypothesis that phosphorus deficiency had developed at this stage, seven of these stands were split for phosphorus application (100 Kg Pha^{-1}) in 1984. In 1985, roots from these stands were again sampled. The application of P-fertiliser induced a significant reduction in ^{32}P uptake by the roots, demonstrating a likely growth response of the trees to the

fertiliser. In September, 1986, two full growing-seasons after fertilisation, the diameter at breast height (DBH) of the trees at the seven stands were measured; fertilised trees of all stands showed significant increases in DBH, ranging from 0.5 to 2.5 cm greater than those of trees not fertilised; four plots were more than 1 cm and three more than 2 cm DBH greater than unfertilised plots. The conclusion was that the bioassay may provide a means of predicting potential growth response. This experiment has the advantage of making relative comparisons. This kind of experiment carried out over a range of sites may provide a set of values of ^{32}P uptake providing the standards necessary to compare with a site under test, but this necessitates the establishment of the long-term field trials previously described.

Routine use of the bioassay may be hindered by practical limitations. The fluctuations in root activity following excision (Chapter 2) and the difficulty in predicting when a 'steady state' may be realised, since the uptake is dependent on the preceeding metabolic activity of the tree, may mean that roots would have to be sampled and assayed within a fixed time period from excision, preferably within 72 hours. Other methods currently used for nutrient assessment have the advantage that soils or plant tissues may be collected, dried and stored until they can be analysed. As the uptake of ^{32}P is metabolically mediated, drying is not possible. Hence, the bioassay could not be treated in the same way as the large scale sampling programmes currently carried out for foliar analysis, whereby foliage samples are collected from throughout the U.K. and sent to a central analytical point. With the bioassay, it would be necessary to have analytical facilities close to the point of sampling. However, this problem is probably offset by the ease of sampling roots for the bioassay over collection of top whorl foliage samples for analysis. This is particularly so for the stands where the bioassay is likely to be of most value; in closed canopy, established Sitka spruce stands of moderate to high productivity which are apparently free of nutrient deficiency but where a response to phosphorus fertiliser is possible (McIntosh, 1984).

In conclusion, to what extent have the initial objectives been achieved ?

The primary objective, to investigate the potential of the bioassay as an indicator of trees' phosphorus status has been substantiated throughout the study. The project has confirmed the rapidity and sensitivity of the bioassay in the detection of phosphorus deficiency in comparison with direct analytical methods.

If the second and third objectives are considered together, a variation in root response is influenced by several factors, most importantly seasonality, but the bioassay appears to use the plant as an integrator of soil properties and physiological activity which influence trees' nutritional status and which are simultaneously influenced by factors such as seasonality. Therefore, it is possible to identify standardised sampling times and positions.

However, the sensitivity of the bioassay, being linked to the metabolic state of the tree, suggests that it may be difficult to determine critical values of ^{32}P uptake which would indicate a probable response to P fertiliser application. If the bioassay is to be a workable predictor of fertiliser response, it will be necessary to ascertain whether or not the variability over several seasons, in several sites will permit the definition of these critical values. The best way to investigate this would be to use the method adopted by Harrison *et al* (1987, in press) described on page 98. This approach would validate the predictive use of the bioassay with respect to growth response following phosphorus fertilisation. The variability in response would need to be examined by testing the bioassay in this manner in stands growing on sites of different soil type and altitude, and of various species and ages over a minimum of two years, sampling at regular intervals.

REFERENCES

ALDHOUS, J.R. (1962). Provenances of Sitka spruce: an account of the nursery stage of experiments sown in 1958. Rep. Forest Res., Lond. 1960/1961, 147-154.

ALDHOUS, J.R. (1975). Forestry Commission Bulletin *43* 'Nursery Practice' HMSO, London.

ALEXANDER, M. (1977). Introduction to Soil Microbiology. Wiley, New York.

ALLEN, S.E., GRIMSHAW, H.M., PARKINSON, J.A. & QUARMBY, C. (1974). Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford.

ARNON, D.I. (1953). The physiology and biochemistry of phosphorus in green plants. In: Soil and Fertiliser Phosphorus in Crop Nutrition (Eds. W. H. Pierre and A. G. Norman) pp. 1-42. Academic Press, New York.

BALL, D.F. & WILLIAMS, W.M. (1968). Variability of soil chemical properties in two uncultivated brown earths. J. Soil Sci. *19*, 379-391.

BINNS, W.O. (1975). Forestry Commission Leaflet *63*. Fertilisers in the forest : a guide to materials. HMSO, London.

BINNS, W.O., MAYHEAD, G.J. & MACKENZIE, J.M. (1980). Forestry Commission Leaflet *76*. Nutrient deficiencies of conifers in British forests. HMSO, London.

BLYTH, J.F. & MACLEOD, D.A. (1978). The significance of soil variability for forest soil studies in North-east Scotland. J. Soil Sci. *29*, 419-430.

BOGGIE, R. (1970). Moisture characteristics of some peat-sand mixtures. Scientific Horticulture *22*, 87-91.

BOWEN, G.D. (1970). Effects of soil temperature on root growth and on phosphate uptake along *Pinus radiata* roots. Aus. J. Soil Res. *8*, 31-42

BOWEN, G.D. (1971). Early detection of phosphate deficiency in plants. Communciations in Soil Science and Plant Analysis *1*, 293-298.

BOWEN, G.D. (1984). Tree roots and the use of soil nutrients. In: Nutrition of Plantation forests (Ed. Bowen G.D. and Nambiar, E.K.S.) Academic Press.

BOWEN, G.D. & ROVIRA, A.D. (1967). Phosphate uptake along attached and excised wheat roots measured by an automatic scanning method. Aus. Jour. Biol. Sci. *20*, 369-378.

BOWEN, G.D. & THEODOROU, C. (1967). Studies on phosphate uptake by mycorrhizas. XIV IUFRO-Cong., Sect. 24, Munchen, 116-138.

BOWMAN, J.C. (1978). Balance sheet for phosphorus in the UK. In: Phosphorus in the Environment : its Chemistry and Biochemistry. pp. 243-253. CIBA Foundation Symposium 57, Pub. Elsevier.

CARLYLE, J.C. (1984). The Nitrogen Economy of Larch:Spruce Mixtures. Ph.D. Thesis, Univ. of Edinburgh.

CENTRE FOR AGRICULTURAL STRATEGY (1978). 'Phosphorus : A resource for UK agriculture'. Centre for Agricultural strategy, Report 2,Reading.

CHAPIN, F. S III & HOLLEMAN, D.F. (1974). Radioassay of ^{32}P in intact plant roots using Cerenkov radiation detection. Int. Jour. Applied Radiation & Isotopes *25*, 568-570.

CHAPIN, F.S. III & BLOOM, A. (1976). Phosphate absorption : adaptation of tundra germinoids to a low temperature, low phosphorus environment. Oikos *27*, 111-121.

CHAPIN, F.S. III, BARSDATE, R.J. & BAREL, D. (1978). Phosphorus Cycling in Alaskan coastal tundra : a hypothesis for the regulation of nutrient cycling. Oikos *31*, 189-199.

COLE, C.V., INNIS, G.S. & STEWART, J.W.B. (1977). Simulation of phosphorus cycling in semi-arid grasslands. Ecology *58*, 1-15

COUTTS, M.P. & PHILIPSON, J.J. (1976). The influence of mineral nutrition on the root development of trees. I. The growth of Sitka spruce with divided root systems. J. Exp. Bot. *27*, 1102-1111.

COUTTS, M.P. & PHILIPSON, J.J. (1978). Tolerance of tree roots to water-logging. I. Survival of Sitka spruce and lodgepole pine. New Phytol. *80*, 63-69.

CROOKE, W.M. & SIMPSON, W.E. (1971). Determination of ammonium in kjeldahl digests of crops by an automated procedure. J. Sci. Fd. Agric. *22*, 9-10.

CUTTLE, S.P. (1983). Chemical properties of upland peats influencing the retention of phosphate and potassium ions.Journal of Soil Science *34*, 75-82.

DEANS, J.D. (1979). Fluctuations of the soil environment and fine root growth in a young Sitka spruce plantation. Plant and Soil *52*, 195-208.

DIGHTON, J. & HARRISON, A.F. (1983). Phosphorus nutrition of lodgepole pine and Sitka spruce stands as indicated by a root bioassay. Forestry *56*, 33-43.

DREW, M.C., SAKER, L.R. & ASHLEY, T.W. (1973). Nutrient supply and the growth of the seminal root system in barley. I. The effect of nitrate concentration on the growth of axes and laterals. J. Exp. Bot. *24*, 1189-1202.

EPSTEIN, E., SCHMID, W.E. & RAINS, D.W. (1963). Significance and technique of short-term experiments on solute absorption by plant tissue. Plant & Cell Physiol. *4*, 79-84.

EVERARD, J.E. (1973). Foliar analysis, sampling methods, interpretation and application of results. Quarterly Journal of Forestry *67*, 51-66.

FORD, E.D. & DEANS, J.D. (1977). Growth of a Sitka spruce plantation: spatial distribution and seasonal fluctuations of lengths, weights and carbohydrate concentrations of fine roots. Plant and Soil *47*, 463-485.

FREEZAILAH, B.C.Y. (1974). Climatic influences on the growth and uptake of phosphorus by Sitka spruce. Ph.D. Thesis, University of Edinburgh.

GUPTA, P.L. & RORISON I.H. (1975). Seasonal differences in the availability of nutrients down a podzolic profile. J. Ecol. *63*, 521-534.

HARRISON, A.F. (1979). Variation of four phosphorus properties in woodland soils. Soil Biology and Biochemistry *11*, 393-403.

HARRISON, A.F. & HELLIWELL, D.F. (1979). A bioassay for comparing phosphorus availability in soils. J. Appl. Ecol. *16*, 497-505.

HARRISON, A.F., DIGHTON, J. & SMITH, M.R. (1984). The phosphorus deficiency bioassay : sample and data handling procedures. (Merlewood Research and Development Paper No. 103), Grange-over-Sands: Institute of Terrestrial Ecology.

HARRISON, A.F., DIGHTON, J., TAYLOR, K. & MCDONALD, M.A. (in Press). Phosphorus nutrition of trees and grasses using a root deficiency bioassay.

HENDRICKS, S.B. & DEAN, L.A. (1947). Basic concepts of soil and fertiliser studies with radioactive phosphorus. Soil Sci. Soc. Amer. Proc. *12*, 98-100.

HOAGLAND, D.R. & BROYER, T.C. (1936). General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. *11*, 471-507.

HSU, P.H. (1963). Effect of initial pH, phosphate and silicate on the determination of aluminium with aluminom. Soil Science *96*, 230-238.

HUMPHRIES, E.C. (1950). The absorption of ions by excised root systems. J. Expt. Bot. *1*, 282-300.

INGESTAD, T. (1971). A definition of optimum nutrient requirements in birch seedlings. Physiologia Pl. *24*, 118-125.

JENSEN, P. & PETTERSON, S. (1980). Nutrient uptake in Scots Pine In: Structure and Function of Northern Coniferous Forests. pp. 229-237. Ecological bulletins 32 (T. Persson ed.).

KIMMINS, J.P. & HAWKES, B.C. (1978). Distribution and chemistry of fine roots in a white spruce subalpine fir stand in British Columbia: implications for management. Can. J. For. Res. *8*: 265-279.

LIEFFERS, V.J. & ROTHWELL, R.L. (19870. Rooting of peatland black spruce and tamarack in relation to depth of water table. Can. J. Bot. *65*, 817-821.

LINDMAN, H.R. (1974). Analysis of Variance in Complex Experimental Designs. Pub. W. H. Freeman & Co., San Fransico.

LOWE, L.E. (1972). Aspects of chemical variability in forest humus layers under a mature western hemlock - western red cedar stand. Can. J. For. Res. 2,487-489.

MAFTOUN, M. & PRITCHETT, W.L. (1970). Effects of Added Nitrogen on the Availability of Phosphorus to Slash Pine on Two Lower Coastal Plain Soils. Proc. Soil. Sci. Soc. Am. *34*, 685-690.

MALCOLM, D.C. & CUTTLE, S.P. (1983). The application of fertilisers to drained peat I. Nutrient losses in drainage. Forestry *56*, 155-174.

MAYHEAD, G.J. (1976). Forest fertilising in Great Britain. Proc. of the Fertilizer Society *158*.

MCCLURE, G.W. (1972). Nutrient distribution in root zones. III. Further studies of the effects of phosphorus distribution on corn and wheat. Can. J. Bot. *50*, 2275-2282.

MCCOLL, J.G. (1973). Environmental factors influencing ion transport in a Douglas fir forest soil in Western Washington. J. Ecol.*61*, 71-83.

MCFEE W.W. & STONE, E.L. (1965). Quantity, distribution and variability of organic matter and nutrients in a forest podzol in New York. Soil Sci. Soc. Amer. Proc. *29*, 432-436.

MCINTOSH, R. (1984). Fertiliser experiments in established conifer stands. Forestry Commission Forest Record *127*, HMSO, London.

MENGEL, K. & KIRBY, E.A. (1982). Ch. 9 'Phosphorus' In: Principles of Plant Nutrition, 3rd. ed., Internat. Potash Institute, Bern, Switzerland.

MEJSTRIK, V.K. & KRAUSE, H.H. (1973). Uptake of ^{32}P by *Pinus radiata* roots inoculated with *Suillus luteus* and *Cenococcum graniforme* from different sources of available phosphate. New Phytologist *72*, 137-140.

MICHALIK, I. (1974). The role of the root system in phosphorus uptake and metabolism. In: Structure and Function of Primary Root Tissues. (Ed. by J. Kolek), pp. 447-452, Slovak. Akademy of Science.

MOHREN, G.M.J., VAN DEN BURG, J. & BURGER, F.N. (1986). Phosphorus deficiency induced by nitrogen input in Douglas fir in the Netherlands. Plant and Soil *95*, 191-200.

MILLER, H.G. (1981). Forest fertilisation : some guiding concepts. Forestry *54*, 157-167.

MILLER, H.G. (1982). Diagnosis of nutrient deficiencies and the prescription of fertiliser applications in biomass production. Report for International Energy Agency Planning Group B (JAB-32).

MILLER, H.G. (1984). Dynamics of nutrient cycling in plantation ecosystems. In: Nutrition of Plantation Forests. Ed. by Bowen, G.D. and Nambiar, E.K.S., Academic Press, pp. 53-79.

MILLER, H.G. & WILLIAMS, B.L. (1969). Research on Forest Soils and Tree Nutrition. Rep. For. Res. Lond., 1969, 142-146.

MILLER, R.J., PEVERLY, J.H. & KOEPPE, D.F. (1972). Calcium stimulates ^{32}P accumulation in corn roots. Agronomy Jour. *64*, 262-266.

MORRISON, I.K. (1974). Mineral nutrition of conifers with special reference to nutrient status interpretation : A review of the literature. Can. Forestry Service, Publ. 1343.

MURPHY, J. & RILEY, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chim. Acta. *27*, 31-36.

NEILSEN, K.F. , HALSTEAD, R.L. & MACLEAN, A.J. (1960). Effects of

soil temperature on the growth and chemical composition of lucerne. Proc. 8th Int. Grassland Congr., 287-292.

OWENS, J.N. & MOLDER, M. (1976). Bud development in Sitka spruce I. Annual growth cycle of vegetative buds and shoots. Can. J. Bot. *54*, 313-325.

PYATT, D.G. (1970). Soil Groups of Upland Forests. For. Comm. Record *72*, HMSO, London.

QUESNEL, H.J. & LAVKULICH, L.M. (1980). Nutrient variability of forest floors near Port Hardy, British Columbia. Can. J. Soil. Sci. *60*, 565-573.

RITCHIE, C.A. & HINCKLEY, T.M. (1975). The pressure chamber as an instrument for ecological research. Adv. in Ecol. Res. *9*, 65-254.

ROBERTS, J. (1976). A study of root distribution and growth in a *Pinus sylvestris* L. (Scots pine) plantation in East Anglia. Plant and Soil *44*, 607-621.

SCHEFFE, H.A. (1959). The Analysis of Variance. New York: Wiley.

SIMPSON, K. (1960). Effect of soil temperature and moisture on the uptake of phosphorus by oats. J. Sci. Fd. Agric. *11*, 449-456.

SIMPSON, K. (1965). The significance of effects of soil moisture and temperature on phosphorus uptake. Tech. Bull. Minist. Agric. Fish Fd. *13*, 19-29.

SPINKS, J.W. & BARBER, S.A. (1947). Study of fertiliser uptake using radioactive phosphorus. Sci. Agr. *27*, 145-146.

STEWART, J.W.B. & MCKERCHER, R.B. (1982). Phosphorus cycle. In: Exp. Microbial Ecology. Eds. R.G. Burns & J. H. Slater, pp 221-238. Blackwell Scientific Publications, Oxford.

TABER, H.G. & MCFEE, W.W. (1972). Nitrogen influence on phosphate uptake by *Pinus radiata*. Forest Science *18*, 126-132.

TAYLOR, G.G.M. (1970). Ploughing Practice in the Forestry Commission. For. Comm. For. Record *73*, HMSO, London.

TITUS, B.D. (1985). Nutrient Dynamics of Sitka spruce stands after clearfelling on peaty gley soils. Ph.D. Thesis, Univ. of Edinburgh,1985.

TOUZET, G., HEINRICH, J.C. & NOHAN, I. (1969). Diagnostic foliare et vitesse de croissance. Afocel 1969, 18-59.

VAN DEN DRIESSCHE, R. (1974). Prediction of mineral nutrient

status of trees by foliar analysis. Bot. Rev. *40*, 347-394.

VERESOGLOU, D.S. & FITTER, A.H. (1984). Spatial and temporal patterns of growth and nutrient uptake of five co-existing grasses. J. Ecol. *72*, 259-272.

WEETMAN, G.F. (1981). Predicting fertiliser needs and responses by soil and tissue tests. Invited paper for the IUFRO World Congress, Sept. 1981, Kyoto, Japan. Forestry Abstracts *44* and unpublished manuscript.

WILLIAMS, R.J.P. (1978). 'Phosphorus biochemistry'. In: Phosphorus in the Environment : Its Chemistry and Biochemistry. pp. 95-108. Ciba Foundation Symposium. 57. Pub. Elsevier.

ZEHETMAYR, J.W.L. (1954). Experiments in tree planting on peat. For. Comm. Bulletin *22*, HMSO, London.

ADDITIONAL REFERENCES

CARTWRIGHT, B. (1972). The effect of phosphate deficiency on the kinetics of phosphate absorption by sterile excised barley roots, and some factors effecting the ion uptake efficiency of roots. Comm. Soil Sci. Pl. Anal. *3*, 313-322.

DREW, M.C., SAKER, L.R., BARBER, S.A. and JENKINS, W. (1984). Changes in the kinetics of phosphate and potassium absorption is nutrient-deficient barley roots measured by a solution-depletion technique. Planta *160*, 490-499.

FITTER, A.H. & HAY, R.K.M. (1987). Environmental Physiology of Plants (2nd Ed.). Academic Press, London.

GLASS, A.D.M. (1978). Regulation of potassium influx into intact roots of barley by internal potassium levels. Can. J. Bot. *56*, 1759-1764.

LEE, R.B. (1982). Selectivity and kinetics of ion uptake by barley plants following nutrient deficiency. Ann. Bot. *50*, 429-449.

LEE, R.B. & RATCLIFFE, R.G. (1983). Phosphorus nutrition and the intracellular distribution of inorganic phosphate in pea root tips: a quantitative study using ^{31}P NMR. J. Exp. Bot. *34*, 1222-1244.

APPENDICES

APPENDIX 2

Mean uptake after storage (pg ^{32}P mg root $^{-1}$) mean values (n = 10) and (95 % confidence limits).

Interval (hrs)	May 1984	August 1985
4	20.3 (12.2)	49.0 (35.0)
8	15.7 (6.8)	27.7 (18.5)
12	25.6 (16.2)	30.7 (11.8)
24	41.0 (17.6)	21.6 (10.2)
36	-	37.6 (19.5)
48	61.5 (46.7)	56.8 (25.3)
60	-	66.4 (41.2)
72	47.1 (32.2)	45.6 (27.4)
84	-	41.9 (23.6)
96	-	50.0 (30.4)
103	-	49.3 (18.7)
168	-	51.9 (33.8)
376	-	59.0 (39.3)
484	-	46.2 (28.9)
676	-	41.2 (12.9)

APPENDIX 3A

Root collar diameter (mm). Mean values (n = 10) and (95 % confidence limits).

Treatment **Species**	**P0**	**P5**	**P10**	**P20**	**P50**
Larch	4.49(0.40)	4.91(0.18)	4.43(0.36)	4.64(0.53)	4.67(0.32)
Pine	4.33(0.36)	4.01(0.27)	4.34(0.41)	4.59(0.21)	4.45(0.26)
Spruce	3.68(0.19)	4.11(0.21)	4.06(0.30)	4.29(0.38)	4.17(0.34)

Analysis of Variance

Source of Variation	SSQ	DF	MSQ	F
Main Effects	10.104	6	1.684	7.919***
Treatment	2.095	4	0.524	2.463*
Species	8.009	2	4.005	18.831***
2-Way Interactions	3.256	8	0.407	1.914 ns
Tr x Sp	3.256	8	0.407	1.914 ns
Explained	13.360	14	0.954	4.487***
Residual	28.710	135	0.213	
Total	**42.070**	**149**	**0.282**	

APPENDIX 3B

^{32}P uptake (pg ^{32}P mg $^{-1}$ root). Mean values (n = 10) and 95 % confidence limits).

Treatment **Species**	**P0**	**P5**	**P10**	**P20**	**P50**
Larch	47.0(24.6)	35.5(11.7)	28.6(12.3)	26.4(12.4)	20.2(8.1)
Pine	53.6(11.8)	45.1(17.2)	14.2(3.3)	12.3(2.7)	10.5(3.0)
Spruce	103.2(38.2)	37.2(12.8)	20.1(10.3)	14.1(3.6)	13.8(2.8)

Analysis of Variance

Source of Variation	**SSQ**	**DF**	**MSQ**	**F**
Main Effects	61834.29	6	10305.72	23.8***
Treatment	59031.77	4	14757.94	34.1***
Species	2802.52	2	1401.26	3.2*
2-Way Interactions	19308.52	8	2413.57	5.6***
Tr x Sp	19308.52	8	2413.57	5.6***
Explained	81142.81	14	5795.91	13.4***
Residual	58467.94	135	433.10	
Total	**139610.75**	**149**	**936.99**	

APPENDIX 3C

Total P (%) in needles. Mean values (n = 10) and (95 % confidence limits)

Treatment Species	P0	P5	P10	P20	P50
Larch	0.27(0.07)	0.27(0.08)	0.39(0.14)	0.49(0.13)	0.82(0.20)
Pine	0.10(0.01)	0.16(0.06)	0.15(0.01)	0.24(0.07)	0.36(0.06)
Spruce	0.19(0.04)	0.29(0.08)	0.33(0.04)	0.41(0.12)	0.57(0.14)

Analysis of Variance

Source of Variation	SSQ	DF	MSQ	F
Main Effects	4.453	6	0.742	39.9***
Treatment	2.904	4	0.726	39.0***
Species	1.549	2	0.774	41.6***
2-Way Interactions	0.403	8	0.050	2.7**
Tr x Sp	0.403	8	0.050	2.7**
Explained	4.856	14	0.347	18.7***
Residual	2.511	135	0.019	
Total	**7.368**	**149**	**0.049**	

APPENDIX 3D

Total N (%) in needles. Mean values (n = 10) and (95 % confidence limits).

Treatment / **Species**	**P0**	**P5**	**P10**	**P20**	**P50**
Larch	1.07(0.27)	1.03(0.46)	1.03(0.21)	0.95(0.13)	0.98(0.15)
Pine	0.79(0.07)	0.81(0.06)	0.81(0.11)	0.80(0.15)	0.88(0.10)
Spruce	0.77(0.08)	0.77(0.08)	0.74(0.16)	0.88(0.22)	0.76(0.09)

Analysis of Variance

Source of Variation	**SSQ**	**DF**	**MSQ**	**F**
Main Effects	1.526	6	0.254	3.77**
Treatment	0.005	4	0.001	0.02 ns
Species	1.521	2	0.761	11.27***
2-Way Interactions	0.275	8	0.034	0.51 ns
Tr x Sp	0.275	8	0.034	0.51 ns
Explained	1.801	14	0.129	1.91*
Residual	9.112	135	0.067	
Total	**10.913**	**149**	**0.073**	

APPENDIX 3E

Total K (%) in needles. Mean values (n = 10) and (95 % confidence limits).

Treatment **Species**	**P0**	**P5**	**P10**	**P20**	**P50**
Larch	2.37(0.64	2.92(0.26)	2.71(0.84)	3.38(0.72)	3.05(0.45)
Pine	0.88(0.10)	1.24(0.27)	1.00(0.13)	1.01(0.08)	1.17(0.23)
Spruce	1.52(0.31)	1.51(0.16)	1.44(0.18)	1.46(0.23)	1.51(0.16)

Analysis of Variance

Source of Variation	**SSQ**	**DF**	**MSQ**	**F**
Main Effects	94.092	6	15.682	51.46***
Treatment	2.842	4	0.711	2.33 ns
Species	91.250	2	45.625	149.72***
2-Way Interactions	3.760	8	0.470	1.54 ns
Tr x Sp	3.760	8	0.470	1.54 ns
Explained	97.853	14	6.989	22.94***
Residual	41.140	135	0.305	
Total	**138.992**	**149**	**0.933**	

APPENDIX 3F

Total Ca (%) in needles. Mean values (n = 10) and (95 % confidence limits).

Treatment Species	P0	P5	P10	P20	P50
Larch	0.38(0.08)	0.44(0.13)	0.37(0.12)	0.32(0.07)	0.37(0.10)
Pine	0.26(0.05)	0.25(0.03)	0.28(0.03)	0.32(0.07)	0.31(0.04)
Spruce	0.24(0.05)	0.22(0.05)	0.26(0.02)	0.24(0.05)	0.29(0.03)

Analysis of Variance

Source of Variation	SSQ	DF	MSQ	F
Main Effects	0.442	6	0.074	7.62***
Treatment	0.023	4	0.006	0.61 ns
Species	0.418	2	0.209	21.63***
2-Way Interactions	0.132	8	0.017	1.71 ns
Tr x Sp	0.132	8	0.017	1.71 ns
Explained	0.574	14	0.041	4.24***
Residual	1.305	135	0.010	
Total	**1.879**	**149**	**0.013**	

APPENDIX 3G

Total Mg (%) in needles. Mean values (n = 10) and (95 % confidence limits).

Treatment **Species**	**P0**	**P5**	**P10**	**P20**	**P50**
Larch	0.28(0.08)	0.26(0.07)	0.27(0.08)	0.22(0.05)	0.26(0.04)
Pine	0.16(0.03)	0.21(0.04)	0.17(.03)	0.20(0.04)	0.21(0.04)
Spruce	0.20(0.03)	0.17(0.03)	0.22(0.03)	0.20(0.04)	0.21(0.04)

Analysis of Variance

Source of Variation	**SSQ**	**DF**	**MSQ**	**F**
Main Effects	0.139	6	0.023	5.41***
Treatment	0.010	4	0.002	0.57 ns
Species	0.130	2	0.065	15.08***
2-Way Interactions	0.049	8	0.006	1.42 ns
Tr x Sp	0.049	8	0.006	1.42 ns
Explained	0.188	14	0.013	3.13***
Residual	0.580	135	0.004	
Total	**0.768**	**149**	**0.005**	

APPENDIX 3H

Total P (%) in roots. Mean values (n = 10) and (95 % confidence limits).

Treatment Species	**P0**	**P5**	**P10**	**P20**	**P50**
Larch	0.12(0.02)	0.10(0.01)	0.11(0.02)	0.12(0.02)	0.12(0.02)
Pine	0.14(0.03)	0.20(0.13)	0.20(0.04)	0.23(0.04)	0.29(0.04)
Sprice	0.12(0.02)	0.16(0.04)	0.15(0.02)	0.18(0.04)	0.19(0.04)

Analysis of Variance

Source of Variation	**SSQ**	**DF**	**MSQ**	**F**
Main Effects	0.309	6	0.052	14.01***
Treatment	0.089	4	0.022	6.05***
Species	0.220	2	0.110	29.95***
2-Way Interactions	0.056	8	0.007	1.90 ns
Tr x Sp	0.056	8	0.007	1.90 ns
Explained	0.365	14	0.026	7.09***
Residual	0.497	135	0.004	
Total	**0.862**	**149**	**0.006**	

APPENDIX 3I

Total N (%) in roots. Mean values (n = 10) and (95 % confidence limits).

Treatment Species	P0	P5	P10	P20	P50
Larch	0.84(0.15)	0.84(0.13)	0.81(0.13)	0.82(0.11)	0.79(0.13)
Pine	0.52(0.07)	0.57(0.03)	0.57(0.07)	0.63(0.04)	0.67(0.05)
Spruce	0.71(0.10)	0.71(0.06)	0.80(0.11)	0.82(0.10)	0.81(0.08)

Analysis of Variance

Source of Variation	SSQ	DF	MSQ	F
Main Effects	1.526	6	0.254	13.97***
Treatment	0.112	4	0.028	1.54 n s
Species	1.413	2	0.707	38.81***
2-Way Interactions	0.154	8	0.019	1.06 ns
Tr x Sp	0.154	8	0.019	1.06 ns
Explained	1.680	14	0.120	6.59***
Residual	2.458	135	0.018	
Total	**4.138**	**149**	**0.028**	

APPENDIX 3J

Total K (%) in roots. Mean values (n = 10) and (95 % confidence limits).

Treatment **Species**	**P0**	**P5**	**P10**	**P20**	**P50**
Larch	0.54(0.11)	0.43(0.18)	0.55(0.07)	0.53(0.14)	0.50(0.09)
Pine	1.17(0.19)	1.10(0.19)	1.18(0.15)	1.20(0.24)	1.27(0.11)
Spruce	0.60(0.09	0.61(0.11)	0.56(0.14)	0.72(0.11)	0.62(0.06)

Analysis of Variance

Source of Variation	**SSQ**	**DF**	**MSQ**	**F**
Main Effects	13.162	6	2.194	57.99***
Treatment	0.188	4	0.047	1.24 ns
Species	12.974	2	6.487	171.49***
2-Way Interactions	0.210	8	0.026	0.70 ns
Tr x Sp	0.210	8	0.026	0.70 ns
Explained	13.373	14	0.955	25.25***
Residual	5.107	135	0.038	
Total	**18.479**	**149**	**0.124**	

APPENDIX 3K

Total Ca (%) in roots. Mean values (n = 10) and (95 % confidence limits).

Treatment Species	P0	P5	P10	P20	P50
Larch	0.28(0.05)	0.31(.05)	0.28(0.04)	0.30(0.04)	0.27(0.05)
Pine	0.25(0.04)	0.24(0.03)	0.26(0.05)	0.29(0.05)	0.28(0.05)
Spruce	0.31(0.06)	0.32(0.02)	0.37(0.07)	0.31(0.04)	0.35(0.05)

Analysis of Variance

Source of Variation	SSQ	DF	MSQ	F
Main Effects	0.130	6	0.022	4.94***
Treatment	0.009	4	0.002	0.50 ns
Species	0.121	2	0.061	13.80***
2-Way Interactions	0.049	8	0.006	1.39 ns
Tr x Sp	0.049	8	0.006	1.39 ns
Explained	0.179	14	0.013	2.91***
Residual	0.592	135	0.004	
Total	**0.771**	**149**	**0.005**	

APPENDIX 3L

Total Mg (%) in roots. Mean values (n = 10) and (95 % confidence limits).

Treatment Species	P0	P5	P10	P20	P50
Larch	0.10(0.01)	0.10(0.02)	0.10(0.01)	0.10(0.01)	0.09(0.00)
Pine	0.10(0.02)	0.08(0.01)	0.10(0.01)	0.09(0.01)	0.09(0.00)
Spruce	0.14(0.03)	0.14(0.01)	0.13(0.02)	0.17(0.02)	0.15(0.01)

Analysis of Variance

Source of Variation	SSQ	DF	MSQ	F
Main Effects	0.084	6	0.014	38.45***
Treatment	0.004	4	0.001	2.44*
Species	0.081	2	0.040	110.47***
2-Way Interactions	0.006	8	0.001	2.06*
Tr x Sp	0.006	8	0.001	2.06*
Explained	0.090	14	0.006	17.66***
Residual	0.049	135	0.000	
Total	**0.140**	**149**	**0.001**	

APPENDIX 4A

^{32}P uptake over the sampling period at Wauchope (pg ^{32}P mg root^{-1}). Mean values (n = 50) and (95 % confidence limit).

	Treatment		
Month	**P**	**N**	**O**
A	56.3(17.1)	90.4(41.4)	95.8(21.9)
M	72.1(11.2)	78.7(19.2)	80.1(22.3)
J	84.4(19.4)	91.7(34.2)	108.7(31.4)
J	116.2(40.4)	196.3(98.6)	157.2(61.8)
A	43.9(15.0)	104.8(71.2)	108.5(52.5)
S	154.3(39.1)	205.7(70.1)	219.9(61.5)
0	89.6(33.6)	217.4(70.5)	174.2(65.0)
N	46.2(2.9)	45.5(2.7)	45.3(2.1)
D	82.6(22.1)	160.0(54.8)	174.9(53.5)
F	110.6(23.1)	153.8(45.8)	167.6(61.6)
M	47.4(12.9)	134.6(69.4)	166.3(108.1)
A	148.5(39.2)	233.0(95.3)	204.3(62.1)

APPENDIX 4B

Composition of University of California Mix D2 Compost

50:50 fine sand/horticultural peat mix.

	per 100 l compost
Potassium Nitrate	15 g
Potassium Sulphate	15 g
Superphosphate	150 g
Magnesian Limestone	445 g
Calcium carbonate	150 g
Fritted trace elements	37 g
Ammonium nitrate or blood	150 g

pH 4 - 5.5

APPENDIX 4C

^{32}P uptake at different root temperatures (pg ^{32}P mg root^{-1}). Mean values (n = 9) and (95 % confidence limit)

	Temperature (^{o}C)		
	5	**12**	**20**
Sitka spruce	350.11(100.1)	343.56(101.7)	352.44(136.4)
lodgepole pine	411.56(182.9)	325.33(134.0)	369.22(114.0)

APPENDIX 4D

Difference in ^{32}P uptake by control and treated plants at different degrees of moisture stress (pg ^{32}P mg root^{-1}). Mean values (n = 10) and (95 % confidence limit).

Sitka spruce

pF		
1.5	**1.8**	**2.0**
-2.80(83.9)	11.30(76.4)	100.40(131.0)
2.4	**2.7**	**3.0**
129.22(106.5)	205.78(133.0)	164.50(186.0)

lodgepole pine

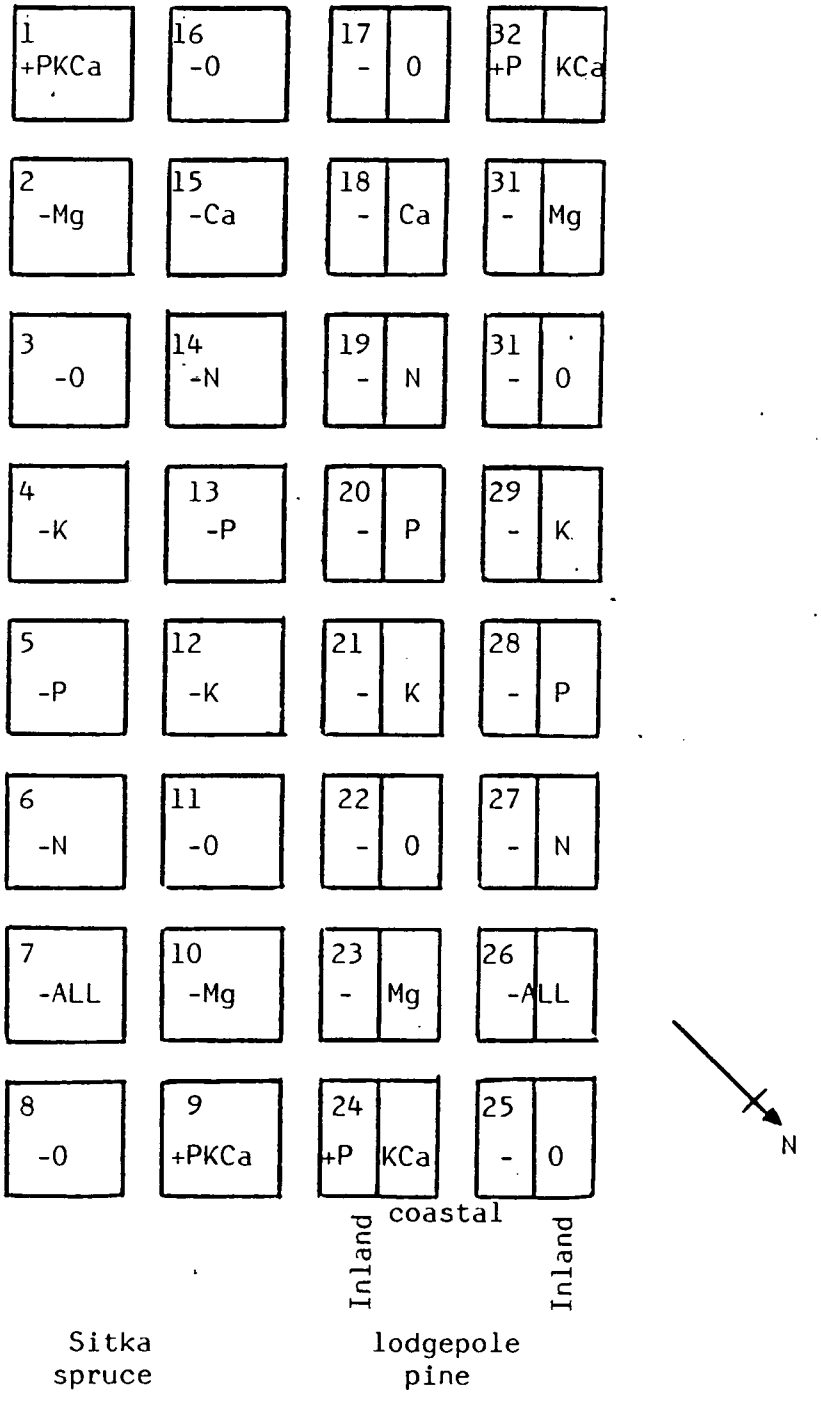
pF		
1.5	**1.8**	**2.0**
-8.10(93.9)	-28.20(98.2)	164.00(173.4)
2.4	**2.7**	**3.0**
181.80(111.8)	158.11(51.8)	201.88(114.7)

APPENDIX 5A

Treatment plots

Plots 1-8)
Plots 25-32) p. 67

Plots 17-24) Originally p.67-
Plots 9-16) felled and replanted in 1973



APPENDIX 5B: Fertiliser (Kg element ha^{-1}) and herbicide applications to plots.

PLOT	+PKCA	-P
3/67	Paraquat	Paraquat
3/67	Planting	Planting
5/67	$P_{42}K_{84}Ca_{68}$	$N_{84}K_{84}Ca_{169}Mg_{56}$
6/68	"	"
6/69	$P_{84}K_{168}Ca$	$N_{168}K_{168}Ca_{339}Mg_{112}$
8/69	2,4-D	2, 4-D
6/70	$P_{84}K_{168}Ca_{136}$	$N_{168}K_{168}Ca_{339}Mg_{112}$
6/71	Cu_{75} to 1/2 plot	-
6/73	$P_{84}K_{168}Ca_{136}$	$N_{168}K_{168}Ca_{339}Mg_{112}$
5/76	"	"
8/77	-	2, 4-D to 1/2 plot
6/79	$P_{84}K_{168}Ca_{136}$	$N_{168}K_{168}Ca_{339}Mg_{112}$
5/82	"	"

Nutrient	Fertiliser
N	Urea
K	Sulphate of potash
Ca	Ground limestone
Mg	Epsom salt
PKCa	Fisons 48

APPENDIX 5C: ANOVA of Eddleston data

Source of Variation	DF	SSQ	% SSQ	MSQ	VR	
Species	1	33,150	0.49	33,150	4.263	-
Fertiliser	1	545,064	8.02	545,064	70.090	-
Position	2	183,904	2.71	91,952	11.824	***
Time	3	446,142	6.56	148,714	19.123	***
Species x Fertiliser	1	388,456	5.71	388,456	49.952	-
Species x Position	2	31,699	0.47	15,849	2.038	ns
Fertiliser x Position	2	37,186	0.55	18,593	2.391	ns
Species x Time	3	521,131	7.67	172,710	22.337	***
Fertiliser x Time	3	800,550	11.78	266,850	34.314	***
Position x Time	6	109,824	1.62	18,304	2.354	ns
Species x Fertiliser x Position	2	22,311	0.33	11,156	1.434	ns
Species x Fertiliser x Time	3	115,151	1.69	38,384	4.936	*
Species x Position x Time	6	85,968	1.26	14,328	1.842	ns
Fertiliser x Position x Time	6	71,053	1.05	11,842	1.523	ns
Residual	438	3,406,168	50.11	7,777		
Total	479	6,797,756	100.00	14,192		

APPENDIX 5D: ^{32}P uptake (pg ^{32}P mg root^{-1}) from different sampling positions in different months.
Mean values (n = 10) and (95 % C.I.)

Sitka spruce			
+PKCa			
Month	**Furrow**	**Flat**	**Ridge**
May	209.20(89.82)	174.40(58.70)	212.20(76.40)
July	44.90(16.76	58.00(63.32)	59.70(63.89)
September	131.70(73.71)	147.50(143.24)	139.60(137.74)
February	108.60(84.92)	155.20(170.39)	141.30(76.16)
-P			
May	137.70(33.09)	156.30(74.30)	188.10(129.46)
July	259.00(178.86)	363.90(188.39)	371.30(194.34)
September	285.00(96.60)	286.80(181.85)	259.20(137.61)
February	246.40(52.08)	273.00(150.64)	247.10(113.15)
lodgepole pine			
+PKCa			
May	192.50(50.38)	319.20(119.94)	341.30(189.77)
July	48.20(28.10)	40.70(30.40)	49.30(30.74)
September	183.10(113.98)	274.60(247.36)	173.10(112.40)
February	158.50(119.32)	159.00(161.88)	126.10(150.58)
-P			
May	184.20(33.04)	228.10(113.68)	286.20(144.46)
July	61.40(49.09)	142.60(137.14)	218.10(119.34)
September	136.40(85.96)	231.10(179(30)	266.70(288.78)
February	141.60(74.42)	170.40(100.64)	124.80(83.88)

APPENDIX 5E: ^{32}P-uptake (pg ^{32}P mg root^{-1}) by split roots differentially supplied with P
(Mean values (n = 22) and (95 % C.I.)).

Sitka spruce	
P0/0	169.19 (90.12)
P0/5	157.24 (142.42)
P0/10	91.58 (53.48)
P0/20	81.01 (44.57)
P0/50	61.95 (38.09)
P5/0	127.24 (89.42)
P10/0	77.29 (43.49)
P20/0	68.38 (44.99)
P50/0	74.48 (44.81)
P50/50	76.91 (44.49)
lodgepole pine	
P0/0	127.76 (54.94)
P0/5	101.71 (47.81)
P0/10	86.76 (49.24)
P0/20	77.81 (31.50)
P0/50	70.57 (40.27)
P5/0	120.38 (53.50)
P10/0	74.24 (43.71)
P20/0	84.00 (38.30)
P50/0	51.48 (23.57)
P50/50	47.57 (17.64)